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Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.01/A1

Topic: A.04. Transplantation and Regeneration

Support: Samsung Science and Technology Foundation (SSTF-BA1301003)
National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2016R1A3B1905982)

Title: Increased ER-mitochondria tethering promotes axon regeneration

Authors: *S. LEE[1,2], W. WANG[1,2,3], J. HWANG[4], U. NAMGUNG[4], K.-T. MIN[1,2];
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Abstract: Molecular tethering between endoplasmic reticulum (ER) and mitochondria plays key roles in calcium buffering, lipid and ion exchange, and mitochondrial dynamics. However, making ER-mitochondria connection and its physiological roles in neurons are still unclear. Translocation of ER and mitochondria to the site of axon injury has been shown to facilitate axonal regeneration; however, the existence and physiological importance of ER-mitochondria tethering in the injured axons are unknown. Here, we show that glucose-regulated protein 75 (Grp75), a protein linking ER to mitochondria, is locally translated after delivering axonal injury. We find that overexpression of Grp75 in primary neurons increases ER-mitochondria tethering to promote regrowth of injured axons. Promoted contact between ER and mitochondria results in elevation of mitochondrial Ca^{2+} and ATP generation, thereby promoting regrowth of injured axons. Furthermore, our results demonstrate that overexpression of Grp75 in sciatic nerves of an animal facilitate axonal regeneration and behavioral recovery. Together, our findings suggest that increased ER-mitochondria tethering at axonal injury sites may provide a therapeutic strategy for axon regeneration.

Disclosures: **S. Lee:** None. **W. Wang:** None. **J. Hwang:** None. **U. Namgung:** None. **K. Min:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.02/A2

Topic: A.04. Transplantation and Regeneration

Support: ANPCYT 2017-2020 / 3952
PIP 2017-2019/ 11220170101059CO
UNLP 18/x807 2019-2020
UBA 2018-2021, 20020170100588BA

Title: Adipose-derived mesenchymal stem cells and magnetic nanoparticles: Different tools combined to promote sciatic nerve regeneration after injury

Authors: *P. A. SOTO[1], G. M. PIÑERO[1], V. USACH[1], M. B. FERNÁNDEZ VAN RAAP[2], C. P. SETTON-AVRUJ[1];
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Abstract: Neuropathies are common problems in public health with high prevalence worldwide. Despite the regenerative capability of the peripheral nervous system (PNS), the poor clinical evolution of patients turns these affections into a crippling disease, which is why the development of new regenerative therapies is of great importance. Research in stem cells has recently become an important tool to promote regeneration in different tissues, including the PNS. Adipose-derived mesenchymal stem cells (AdMSC) are multipotent adult stem cells fully investigated as a promising tool to develop regenerative therapies. Wallerian degeneration (WD) is an efficient animal experimental model in mimicking the impact of peripheral nerve lesion to shed light on possible regeneration strategies. AdMSC transplant is a useful tool for regenerative therapies, while magneto targeting has emerged as a nanotechnological strategy to mobilize magnetic nanoparticle (MNP). In this context, the aim of the present work was to test whether magneto targeting can help AdMSC-loaded MNP (AdMSC-MNP) reach specific tissue guided by an external magnetic field and enhance the regenerative ability of AdMSC upon rat sciatic nerve lesion. To test our hypothesis, AdMSC were characterized through immunocytochemistry, western blot and flow cytometry for multipotent cell marker expression. MNP internalization to AdMSC was evaluated through transmission electron microscopy and vibrating sample dc-magnetometry (VSM) experiments. Likewise, confocal microscopy and VSM analyses were performed to evaluate the arrival of AdMSC-MNP at the injured nerve. Finally, cell transplantation effects on nerve regeneration were evaluated both in terms of morphology and conduction through immunofluorescence, western blot and electrophysiological experiments.

Our results show that AdMSC in passage 3 express multipotent markers CD105, CD90 and CD73 but not CD11b or CD45, and can internalize 2 to 4 pg MNP/cell. We demonstrate that AdMSC-MNP supersede AdMSC arrival exclusively at the lesion site and their beneficial effects on sciatic nerve regeneration. In short, our results prove that magneto targeting of AdMSC-MNP constitutes a novel and valuable tool to promote nerve regeneration by enhancing AdMSC arrival at the lesion site.

Disclosures: **P.A. Soto:** None. **G.M. Piñero:** None. **V. Usach:** None. **M.B. Fernández van Raap:** None. **C.P. Setton-Avruj:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.03/DP01/A3

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: A.04. Transplantation and Regeneration

Support: The Company of Biologists Travelling Fellowship Grant - Journal of Experimental Biology "Label-free multiphoton microscopy as a tool for the investigation of developing and regenerating arms in the cephalopod mollusc Octopus vulgaris" – 2019
Association for Cephalopod Research – CephRes, Napoli, Italy
STSM COST Action FA1301 "Label-free multiphoton microscopy as a tool for the investigation of nerve regeneration in the cephalopod mollusc Octopus vulgaris" - 2016

Title: Regeneration in octopus vulgaris: Imaging tools for healing and re-wiring

Authors: *P. IMPERADORE[1,2], O. UCKERMANN[3], R. GALLI[4], M. KIRSCH[3,5], G. FIORITO[2];
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Abstract: Regeneration is a process that restores structure and function of damaged tissues. The PNS of adult mammals retains high regenerative potentials but recovery is often unsatisfactory. Cephalopods, as some other other invertebrates, show the ability to effectively regenerate tissues and parts. Particularly, in *Octopus vulgaris* the pallial nerve and the arm appear to be appealing

models for regenerative studies. Octopuses have two pallial nerves, connecting the brain to the periphery, allowing for the control of respiratory movements and skin patterning. The injury of one nerve leads to loss of control on these functions on one side of the animal. The eight arms, instead, are flexible muscular hydrostats lacking fluid-filled cavities and hard skeletal supports, providing them with high degrees of freedom, useful for exploring the environment and in predation, but also exposing them to plenty of potential damages. Both the above mentioned structures are endowed with the capacity of healing and functionally regenerating after severe injury.
Direct imaging of injured tissues has always represented an extremely advantageous approach in regenerative studies. This technique in cephalopods has been limited by the reduced number of markers commercially available for these organisms. Additionally, antibody staining usually limits the observations to the investigated epitope, leaving in the dark a huge amount of information and events that characterize complex phenomena. New microscopy methods available for vertebrates allow to investigate regenerative events overcoming these issues. Vibrational spectroscopy, for instance, probes vibrational energy levels associated with the chemical bonds in the sample. Additionally, multiphoton microscopy, does not rely on species-related epitopes, thus allowing its use in a species-independent way and facilitating comparison among various animal species. Here we present the results obtained applying these label-free techniques, to the regenerating pallial nerve and arm of *Octopus vulgaris*. The approach allowed the identification of cells and structures usually not revealed through classical staining: hemocytes building up scars and phagocytizing debris (through CARS), degenerating fibers and muscles (TPEF) and the formation of a leading connective tissue bridge (SHG) involved in axons guidance. These provided helpful morpho-chemical information to describe regeneration events, revealing to be species-specific independent and appearing promising for regenerative studies in cephalopods and other non-model species.

Disclosures: P. Imperadore: None. **O. Uckermann:** None. **R. Galli:** None. **M. Kirsch:** None. **G. Fiorito:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.04/A4

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant K01NS105879-01
NIH Grant R01NS041596-11S1
South Carolina Honors College SURF Scholars Research Program

Title: Differential changes to mRNA localization in central sensory axons after peripheral nerve injury

Authors: *T. P. SMITH, J. E. MARRYAT, J. L. TWISS;
Biol. Sci., Univ. of South Carolina, Columbia, SC

Abstract: It is well established that the transport and local translation of mRNAs in axons and dendrites play an important role in growth/regeneration and plasticity. Our lab has shown that the growth associated mRNA growth associated protein-43 (GAP-43) increases in the peripheral axons of dorsal root ganglion (DRG) neurons after peripheral nerve injury and this contributes to increased regenerative capacity of these axons. It has yet to be seen if the transcriptome is altered in the central axons of DRG neurons after peripheral nerve injury. In this study, we hypothesized that a crush injury to the sciatic nerve would result in differentially localized injury and pain associated mRNAs in the centrally projecting axons of these neurons. Using reverse transcriptase droplet digital PCR, we were able to show that 7 days following sciatic nerve crush, GAP-43 and the injury and pain associated mRNAs galanin and calcitonin gene related peptide (CGRP), are significantly increased in the centrally projecting axons compared to the naïve condition. The increase in GAP-43 mRNA persists 21 days following peripheral nerve injury only in the centrally projecting axons. We also show that the transcriptome of the centrally projecting axons is different from the peripherally projecting axons. Surprisingly, we also show that incomplete injury to the sciatic nerve results in differential changes to growth and pain associated mRNAs in DRG neurons. These results show that following peripheral axotomy, the transcriptome, particularly mRNAs involved in regeneration and pain modulation, is differentially altered in the centrally projecting sensory axons potentially providing more insight into the intrinsic growth capacity of these centrally projecting sensory axons as well as the mechanisms involved in the development of neuropathic pain.

Disclosures: T.P. Smith: None. **J.E. Marryat:** None. **J.L. Twiss:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.05/A5

Topic: A.04. Transplantation and Regeneration

Support: Tyrolean Research Fund Grant UNI-0404/1920

Title: Simultaneous downregulation of Sprouty2 and pten promotes axon growth of adult sensory neurons

Authors: S. JAMSUWAN, L. KLIMASCHEWSKI, ***B. HAUSOTT**;
Neuroanatomy, Med. Univ. Innsbruck, Innsbruck, Austria

Abstract: Peripheral nerve injury is common and afflicts individuals at different ages. Although peripheral nerves are provided with the ability to regenerate in response to injuries, the functional outcomes are often poor. Intracellular pathways required for axon regeneration are under the tight control of endogenous inhibitors. The rat sarcoma (RAS)/extracellular-signal regulated kinase (ERK) pathway is regulated by Sprouty (Spry) proteins, whereas the phosphatidylinositol-3-kinase (PI3K)/AKT pathway is under the control of phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Downregulation of Spry2 or PTEN enhances axon growth *in vitro* and *in vivo*. It is now the goal of the present study to analyze the effects of simultaneous downregulation of Spry2 and PTEN on axon growth of adult sensory neurons *in vitro*. We used dissociated adult sensory neuron cultures from wild-type, heterozygous and homozygous Spry2 knockout mice and transfected them with Accell^{TM} siPTEN. PTEN and Spry2 levels were determined by qPCR, immunostaining and western blotting. Axon growth was measured 72h after transfection with siPTEN using MetaMorph morphometry software. Activation of pAKT and pERK was determined by western blotting. Sufficient siRNA induced knockdown of PTEN mRNA and protein was observed after 72h. PTEN was expressed by all subtypes of sensory neurons after 72h in culture, and endogenous PTEN protein levels were reduced during their time in culture. Furthermore, PTEN protein was reduced in cultures from Spry2 knockout mice and Spry2 protein was reduced in response to PTEN knockdown in wild-type and heterozygous Spry2 neuron cultures. Knockdown of PTEN enhanced axonal elongation of neuron cultures from homozygous Spry2 knockout mice whereas axonal branching was less pronounced. Activation of pAKT was enhanced in response to knockdown of PTEN and this effect was stronger in cultures from homozygous Spry2 knockout mice than in wild-type cultures but no difference in the activation of pERK was observed. Together these results demonstrate for the first time a reciprocal regulation of Spry2 and PTEN in adult sensory neurons. Our previous results revealed a branching phenotype of neurons from homozygous Spry2 knockout mice and the knockdown of PTEN reversed this to a more elongative phenotype and enhanced activation of pAKT. These findings indicate that the simultaneous downregulation of Spry2 and PTEN promotes axonal elongation which is required for long distance axon regeneration.

Disclosures: S. Jamsuwan: None. **L. Klimaschewski:** None. **B. Hausott:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.06/A6

Topic: A.04. Transplantation and Regeneration

Support: Foundation Grant

Title: Molecular pathways of facial nerve regeneration following injury

Authors: *C. FANIKU[1], M. ZHANG[1], J. YUAN[1], W. KONG[2], J. PEPPER[1];
[1]Dept. of Otolaryngology, Stanford Sch. of Med., Palo Alto, CA; [2]Inst. for Stem Cell Biol. and Regenerative Med., Stanford Univ., Palo Alto, CA

Abstract: Facial nerve injury results in the marked expansion of a population of endoneurial fibroblasts that are responsive to Hedgehog (Hh) pathway stimulation, as marked by their expression of Hh pathway readout *Gli1* (Dogaru, G., *et al.*, 2018). The function of Hh signaling in this context is unknown, as are the pathway ligands and their cellular presentation. Desert hedgehog (Dhh) signaling is required for normal perineurial development (Parmantier, E., *et al.*, 1999). Therefore, we hypothesize that: 1) Dhh expression within the facial nerve increases after nerve injury, and 2) pathway activity plays a key role in reformulation of the injured facial nerve perineurium by stimulating angiogenesis and cellular migration in the injured tissue. Using the *Dhh*^{CreERT2};*R26*^{tdTomato} transgenic mouse model, we demonstrate that facial nerve Dhh expression increases 7 days after transection injury. Immunohistochemistry of nerve tissue sections using S100beta antibody reveals that the Dhh+ cells within the facial nerve are a subpopulation of Schwann cells. To understand the role of Hh-responsive cells in nerve injury, we exposed cultured facial nerve fibroblasts to a potent Hh pathway agonist, SAG21k. Using live cell imaging, we describe the impact of Hh pathway stimulation on endoneurial fibroblast migration *in vitro*. Gene expression levels of *VEGF-A* and *Angpt 1* were significantly increased in facial nerve fibroblasts exposed to SAG21k versus control at 24 hours, as determined by qPCR. Our findings point to an intriguing and novel role for Dhh signaling in facial nerve injury response in adult mammals. Hh pathway stimulation may direct post-injury angiogenesis and reformation of the perineurium. Given that functional restoration of the perineurium after injury is not understood, further investigation of the role of Hh signaling in the context of facial nerve injury may lead to new treatment strategies.

Disclosures: C. Faniku: A. Employment/Salary (full or part-time):; Stanford University. **M. Zhang:** A. Employment/Salary (full or part-time):; Stanford University. **J. Yuan:** A. Employment/Salary (full or part-time):; Stanford University. **W. Kong:** A. Employment/Salary (full or part-time):; Institute for Stem Cell Biology and Regenerative Medicine Stanford. **J. Pepper:** A. Employment/Salary (full or part-time):; Stanford University.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.07/A7

Topic: A.04. Transplantation and Regeneration

Support: The General Research Fund grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (Ref. No.: 11100417)

Title: Genetic ablation of formin protein accelerates axon regeneration via modulation of microtubule dynamics

Authors: *N. P. B. AU, G. KUMAR, X. WANG, C. H. E. MA;
Dept. of Biomed. Sci., City Univ. of Hong Kong, Kowloon Tong, Hong Kong

Abstract: Peripheral nerve injury (PNI) reactivates the intrinsic growth machinery necessary for axonal outgrowth, which enable axon regeneration after injury. Proximal PNI requires axon regeneration over a long-distance for target reinnervation which usually take months or years to fully extend to distal muscle targets. By the time when axons finally reach their original targets, they failed to re-establish connections and form functional synapses after chronic denervation. This accounts for the poor functional recovery in patients with proximal PNI. We demonstrated that the damaged axons must extend to the distal muscle within a critical period of 35 days in adult mice for complete restoration of motor functions, and by accelerating axonal regrowth it is plausible to promote functional recovery after PNI. Formin proteins are protein superfamily which share highly conserved FH1 and FH2 domains. Ubiquitous expression of formin proteins is detected in both central and peripheral nervous system; however, its functions, especially in regulation of axon regeneration, remain largely unknown. Compelling evidence suggested that both FH1 and FH2 domains in formin proteins can bind to microtubule and modulate its dynamics and stability, which is a crucial determinant for successful axon regeneration. Our pilot study has demonstrated that PNI induced down-regulation of formin protein. *In vivo* silencing of formin protein using target-specific formin-short interfering RNA (formin-siRNA) markedly accelerated axonal regrowth, and promoted sensory and motor functional recovery after sciatic nerve crush injury in adult mice. In the current study, we further tested if complete ablation of formin protein could accelerate axon regeneration after PNI using formin complete knockout mice (formin-KO). Cultured dorsal root ganglion (DRG) neurons prepared from formin-KO mice exhibited significantly longer neurites with more axonal branching compared with their wild-type littermates. We then performed sciatic nerve crush injury on formin-KO mice and assessed the distal extent of axon regeneration using sciatic nerve pinch test. In line with our *in vitro* results, complete ablation of formin protein markedly accelerated axonal regrowth 3 days after crush injury. Sensory and motor functional recovery also markedly improved in formin-KO mice as assessed by an exhaustive list of neurobehavioral and electrophysiological studies after crush injury. Further investigation of molecular mechanisms underlying the formin-mediated microtubule dynamics shed new light in developing novel therapeutic approaches to promote functional restoration in patients with proximal PNI.

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Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.08/A8

Topic: A.04. Transplantation and Regeneration

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 05160126)

Title: Overexpression of basic helix-loop-helix protein promotes axon regeneration after nervous system injuries

Authors: *G. KUMAR[1], N. P. B. AU[1], S. K. CHIU[1], D. H. GESCHWIND[2], G. COPPOLA[2], C. H. E. MA[1];
[1]Dept. of Biomed. Sci., City Univ. of Hong Kong, Kowloon Tong, Hong Kong; [2]Dept. of Psychiatry and Neurology, Semel Inst. for Neurosci. and Human Behavior, UCLA, Los Angeles, CA

Abstract: Injuries to nervous system are devastating that patients often suffer from irreversible and permanent loss of sensory and motor functions after injuries. Mature neurons in central nervous system (CNS) failed to regrow the damaged axons after injury. In contrast, neurons in peripheral nervous system (PNS) can regenerate their injured axons via reactivation of regeneration-associated genes after injury. However, limited motor functional recovery is frequently observed in patients with proximal peripheral nerve injuries (PNI) such as brachial plexus injuries, largely due to the slow regeneration rate (i.e. 1-2mm/day) of peripheral axons after PNI. At the time when the regenerated axons reach their original target at the motor end plates, they failed to reinnervate to form functional synapses after chronic denervation. It has an urge need to develop new therapeutic interventions to promote axonal regrowth and thus functional recovery after PNI. Basic helix-loop-helix (bHLH) protein is a transcription factor that bind to E-box motif CAGCTG of its target gene to regulate gene expression. bHLH protein is involved in regulation of key cellular events including cell proliferation, migration and differentiation. It is widely expressed in forebrain in the developing nervous system but its function in the nervous system, especially its role in axonal outgrowth, remained elusive. Recently, we identified bHLH protein as a key regulator in axon regeneration. Gene silencing of bHLH protein in adult peripheral (i.e. dorsal root ganglion; DRG) neurons markedly reduced axonal regrowth, and delayed sensory and motor functional recovery after PNI. To further define its role in axon regeneration, we overexpressed bHLH protein in adult DRG neurons using adeno-associated virus (AAV) via intrathecal injections. Overexpression of bHLH protein dramatically promoted axonal regrowth as assessed by sciatic nerve pinch test 3 days after sciatic nerve crush injury. More importantly, overexpression of bHLH protein markedly accelerated

sensory and motor functional recovery after sciatic nerve crush injury. To assess whether bHLH protein also promoted axon regeneration after CNS injury, AAV-bHLH was injected intravitreally to transduce bHLH protein in retinal ganglion cells 2 weeks before optic nerve crush injury. Strikingly, we observed robust axon regeneration in AAV-bHLH-treated mice two weeks after optic nerve crush. More in-depth bioinformatics analysis will elucidate the molecular mechanisms underlying bHLH-induced growth-promoting effects, which help identification of small molecules that activate the signalling pathways associated with bHLH overexpression.

Disclosures: G. Kumar: None. **N.P.B. Au:** None. **S.K. Chiu:** None. **D.H. Geschwind:** None. **G. Coppola:** None. **C.H.E. Ma:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.09/A9

Topic: A.04. Transplantation and Regeneration

Support: HMRF 05163296
HMRF 06173706
HMRF 06172326
HMRF 05163156

Title: Human bone marrow-derived Schwann cells seeded and nanofiber-packed conduit for peripheral nerve regeneration

Authors: *D. K.-Y. SHUM[1], S. CAI[1], W.-C. WONG[1], Y.-Y. WONG[1], K.-W. TAM[1], L.-F. KWOK[1], G.-H. SHEA[2], Y.-S. CHAN[1];
[1]Sch. of Biomed. Sci., [2]Dept. of Orthopaedics and Traumatology, The Univ. of Hong Kong, Hong Kong, China

Abstract: For bridging gaps between stumps of severed peripheral nerves, (1) myelinating cells and (2) physical guidance channel are needed. We reported selective expansion of neuro-ectodermal progenitor cells among the human bone marrow stromal cells (BMSCs) for cytokine induction into Schwann cell-like cells and then co-culture with embryonic dorsal root ganglion neurons to accomplish the switch to fate-committed Schwann cells (Cai et al, 2017a). Here we report use of human iPSC-derived sensory neurons (Cai et al., 2017b) as a surrogate for the co-culture to achieve fate-committed human Schwann cells. Following storage under liquid nitrogen for extended periods, the Schwann cells were thawed for use on demand. We seeded the cells into chitosan-based nerve guidance channels for bridging a critical gap in a rat model of sciatic nerve injury; axonal regrowth and remyelination were observable across the gap in two months. Alternatively, we packed genipin-treated, uniaxially aligned chitosan nanofibers into the

guidance channel for the critical gap-bridging experiment. In set-ups without Schwann cell-seeding and one month after bridging, the genipin-treated chitosan nanofibers retained structural integrity, showing early function of a nerve bridge in which (1) Schwann cells adhered to and proliferated along the direction of genipin-treated chitosan nanofibers and (2) axons grew into the conduit directionally guided by the nanofibers. We expect our strategy to support translation into a protocol whereby human bone marrow-derived Schwann cells become available for autologous transplantation and the genipin-treated chitosan nanofibers accelerate axonal regrowth and remyelination. (Supported by HMRF 05163296, 06173706, 06172326 and 05163156)

Disclosures: D.K. Shum: None. **S. Cai:** None. **W. Wong:** None. **Y. Wong:** None. **K. Tam:** None. **L. Kwok:** None. **G. Shea:** None. **Y. Chan:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.10/A10

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R01NS086818

Title: Critical role of monocarboxylate transporter MCT1 in macrophage immunometabolism for recovery from peripheral nerve injury

Authors: *M. K. JHA, Y. LEE, F. YANG, A. HOKE, J. D. ROTHSTEIN, B. M. MORRISON; Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: The remarkable regenerative capacity of the peripheral nervous system (PNS) requires a high metabolic energy demand. Traumatic nerve injury triggers a cascade of events that culminate in a robust infiltrating macrophage-dependent inflammatory reaction, which is indispensable to normal progression of Wallerian degeneration and regeneration. However, the immunometabolism of macrophages in peripheral nerve regeneration has not yet been explored. We had previously published that reducing the primary lactate transporter in the peripheral nerve, monocarboxylate transporter (MCT1), by half in all cells dramatically delayed nerve regeneration. Through careful analysis of cell-specific deletion of MCT1, we have now determined that deletion of MCT1 only in macrophages leads to a similar delay in nerve regeneration following injury, as measured by nerve electrophysiology and histology, and neuromuscular junction re-innervation. MCT1-deficient macrophages *in vitro* have impairments in both mitochondrial oxidative phosphorylation and glycolysis. Classic proinflammatory cytokines are increased and pro-regenerative cytokines are reduced in macrophages *in vivo* following nerve injury and *in vitro*, suggesting that the delayed nerve regeneration is due to

alterations in macrophage phenotype. Detailed transcriptomic, bioenergetic and functional analyses of macrophages with and without MCT1 are ongoing. Our studies in progress on evaluating nerve regeneration in transgenic mice with focal upregulation of MCT1 in macrophages will provide a potential novel therapeutic pathway for accelerating peripheral nerve regeneration.

Disclosures: M.K. Jha: None. **Y. Lee:** None. **F. Yang:** None. **A. Hoke:** None. **J.D. Rothstein:** None. **B.M. Morrison:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.11/A11

Topic: A.04. Transplantation and Regeneration

Support: CIHR 1486745
FoMD
NMHI
QEII Graduate Award

Title: Clustered protocadherins restrict neurite outgrowth during peripheral nerve regeneration

Authors: *R. M. LONG[1], A. CHANDRASEKHAR[2], D. W. ZOCHODNE[3];
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Abstract: Peripheral nerves are at greater risk of damage than the brain or spinal cord. In addition, a range of common conditions generally titled ‘neuropathies’ render axon damage that is disabling and often irreversible. The peripheral nervous system (PNS) has a limited capacity to regenerate but new understanding of its biology may yield insights into better regrowth. During regeneration of sensory axons, fibres must navigate to their target region while coordinating with existing intact axons. In the developing central nervous system (CNS) growing neurons use self-recognition strategies, which in mammals is driven by the clustered protocadherins (Pcdh; Lefebvre et al. 2012). Neurons lacking either the α or γ cluster show dendritic trees with high instances of self-overlap and little complexity (Suo et al. 2012, Ing-Esteves et al. 2018). This effect has been demonstrated in multiple types of CNS neurons, but its expression and function are unknown in the PNS. We suggest that Pcdh clusters participate in the patterning of epidermal re-innervation, and contribute to regenerative success. Pcdh-α and -γ proteins are expressed in the dorsal root ganglion (DRG) cell body, alongside low level axonal expression. We collected DRGs at three timepoints (0, 36, 72h) following a sciatic nerve axotomy and found that the Pcdh-γ mRNA levels decrease in the DRG at 36h, followed by a return to baseline by 72h

($p<0.05$) with a similar trend in protein ($p=0.28$), suggesting sensory neurons may act quickly to restore Pcdh levels following an injury. We knocked down Pcdh-α and/or -γ using siRNA in dissociated adult mouse DRG neuron cultures and conducted a neurite extension analysis after 72h. We observed increased outgrowth following Pcdh-γ knockdown ($p<0.05$), as well as when both clusters were knocked down simultaneously ($p<0.05$) compared to a scrambled control. We observed a similar trend in the Pcdh-α knockdown ($p = 0.09$). These preliminary results indicate that the Pcdh protein may act as a regenerative "brake", with self-recognition restricting outgrowth of sensory neurons during regeneration in order to facilitate structured patterning of skin re-innervation. Taken together, we demonstrate Pcdh expression in the PNS, primarily localized to the DRG sensory neuron perikarya , and these levels respond to peripheral axotomy injury. When Pcdh clusters are knocked down, there is an increase in total neurite outgrowth. This suggests that Pcdh may act as a restrictor for unwarranted sprouting, and may have important implications in manipulating the extent and patterning of peripheral axon regeneration.

Disclosures: R.M. Long: None. **A. Chandrasekhar:** None. **D.W. Zochodne:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.12/A12

Topic: A.04. Transplantation and Regeneration

Support: Materson ERF award

Title: Increased neurite outgrowth in iPSC-derived neurons carrying the met allele of the Val66Met BDNF polymorphism

Authors: *C. MCGREGOR[1,2], J. PHILLIPS[4], J. D. FINAN[4], C. K. FRANZ[1,2,3];
[1]Biologics, Shirley Ryan Abilitylab, Chicago, IL; [2]Physical Med. and Rehabil., [3]Neurol., Northwestern Univ., Chicago, IL; [4]Northshore Univ. Healthsystem, Evanston, IL

Abstract: Every year, over 200,000 peripheral nerve injuries (PNI) occur in the United States. Although injured axons have the potential to regenerate, success is very rare, and over 90% of PNI patients sustain permanent impairments after injury. Recently, there has been interest in how genetic variability in patient populations may affect outcome. A common single nucleotide polymorphism in the brain derived neurotrophic factor (*BDNF*) gene, Val66Met, results in a valine to methionine substitution at the 66th codon of the protein. In a rodent model of this SNP, transgenic mice heterozygous (V/M) or homozygous (M/M) for the Met allele had enhanced peripheral axon regeneration after a sciatic nerve transection compared to wild type (V/V) controls. The purpose of the current study is to use isogenic stem cells to test whether these differences in axon outgrowth can be recapitulated in a human model. Human induced

pluripotent stem cells (iPSCs) from two donors were genotyped to determine expression of the Val66Met SNP. Using the CRISPR-Cas system, these cells were then edited so that all three genotypes, V/V, V/M, and M/M, could be generated from the same parent line. The cells were then differentiated into motoneurons and cortical neurons. Neurite outgrowth was measured 48 hours after plating, and we observed increased neurite outgrowth in iPSC-derived neurons with M/M genotype as compared to V/V.

Disclosures: C. McGregor: None. **J. Phillips:** None. **J.D. Finan:** None. **C.K. Franz:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.13/A13

Topic: A.04. Transplantation and Regeneration

Support: CIHR Grant RES00442537

Title: Electrical stimulation as a conditioning strategy for promoting peripheral nerve regeneration in a distal nerve transfer

Authors: *J.-L. SENGER[1], K. CHAN[3], K. RABEY[1], M. MORHART[1], C. A. WEBBER[2]; [1]Dept. of Surgery, [2]Div. of Anat., Univ. of Alberta, Edmonton, AB, Canada; [3]Univ. Alberta, Edmonton, AB, Canada

Abstract: Background: Injury to the common peroneal nerve (CP) results in foot drop with major impact on patient's function and quality of life. Reinnervating the distal stump of the degenerated common fibular nerve with a branch of the tibial nerve in a distal nerve transfer (DNT) has gained popularity outcomes remain inconsistent due to poor regeneration. We hypothesize that delivering one hour of cconditioning electrical stimulation (CES) 7-days prior to DNT surgery will significantly improve regeneration and functional outcomes. **Methods:** Using a rat model, the common peroneal (CP) nerve was crushed to replicate a traumatic CP nerve injury. CES was delivered to the tibial nerve in half the animals one-week post-injury. Seven days later, a DNT was performed, in which a tibial branch to the lateral gastrocnemius muscle was cut and coapted to the deep peroneal nerve branch to the tibialis anterior muscle. Speed of nerve regrowth was quantified after 2 weeks of regeneration. Motor reinnervation (nerve conduction study, neuromuscular junction analysis and muscle weight) and functional outcomes (kinetic and kinematic studies and skilled locomotion were assessed after 6-10 weeks of regeneration. **Results:** Animals treated with CES prior to DNT had significantly greater regeneration and motor recovery compared to animals treated with surgery alone. Animals treated with CES had axonal extension 7.8 ±0.8 mm compared to 3.1 ± 0.5 mm in the non-conditioned controls ($p<0.001$). By 9 weeks, on gait analysis conditioned animals had significant

improvement in normalization of the vertical peak, braking, and propulsion forces, gait kinematics, and performance on the horizontal ladder test ($p<0.001$ for all comparisons). The tibialis anterior of the affected limb had greater muscle mass, and significantly more reinnervated neuromuscular junctions ($p<0.01$) **Conclusions:** Delivery of CES one week prior to lower limb DNT from the tibial branch to lateral gastrocnemius muscle significantly improved muscle reinnervation in the tibialis anterior muscle and functional recovery. This treatment could potentially benefit patients with CP nerve injuries.

Disclosures: J. Senger: None. **K. Chan:** None. **K. Rabey:** None. **M. Morhart:** None. **C.A. Webber:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.14/A14

Topic: A.04. Transplantation and Regeneration

Support: Craig H. Neilsen Foundation

Title: Phenotypic characterization of human iPSC-derived motor neurons that carry the common val66met single nucleotide polymorphism of brain derived neurotrophic factor gene using multielectrode array and an *in vitro* stretch injury system

Authors: *M. QUEZADA[1], K. COTTON[1], J. K. PHILLIPS[2], J. D. FINAN[2], C. K. FRANZ[3]; [1]Biomed. Engin., Northwestern Univ., Chicago, IL; [2]Northshore Univ. Healthsystem, Evanston, IL; [3]Physical Med. and Rehabil., Shirley Ryan Abilitylab, Chicago, IL

Abstract: Neurotrauma is the most common injury leading to death and long-term disability. Studies on type and severity of neurotrauma have shown the progression of symptoms and recovery are highly variable. Recent clinical observations have made associations between several common genetic variants and neurotrauma outcomes, but these are not capable of establishing cause and effect. Isogenic human induced pluripotent stem cells (hiPSCs) can be used to isolate single genetic variations that influence neurotrauma outcomes. Isogenic lines only differ from its parental line by a targeted mutation of a single gene. As a result, any phenotypic differences observed between the isogenic lines at baseline or under stress can be attributed to the gene mutation. To begin to understand the influence of genetic variance in neurotrauma, we are studying the val66met single nucleotide polymorphism (SNP) in the brain derived neurotrophic factor (*BDNF*) gene. This SNP results in a valine to methionine substitution, which reduces the intracellular sorting and activity dependent release of BDNF. The met allele of this SNP is common in the American population. It generally correlates to poorer neurological health but has been shown to be protective in some conditions like neurotrauma. We have generated

two independent sets of isogenic hiPSCs that differ only by the val/val, val/met and met/met SNPs in the *BDNF* gene. We differentiated the isogenic cell lines into motor neurons (MNs) using an established protocol. The lines did not differ in their capacity to be differentiated in MNs. The hiPSC-MNs were then cultured in 96 well plates with multi-electrode arrays (MEAs). Extracellular voltage recordings were recorded every other day using Maestro Pro (Axion BioSystems, USA). The results show initial firing at day 5 after plating, and initial bursting activity at day 10. Firing rate increased from day 5 to 25 in all lines. However, no statistical significance differences were found in firing rates among the three lines over time. These results suggest there are no baseline differences in the development of electrical activity in these BDNF isogenic hiPSC-MNs. Ongoing studies include measurements of BDNF release from isogenic MNs after electrical or optogenetic stimulation. We are also exploring the effect of a mechanical stretch injury on MN survival, neurite outgrowth, and electrophysiological function between BDNF genotypes. The findings of this research will help understand the mechanisms that contribute to high variability in neurotrauma outcomes and serve as a template for future studies aimed at more personalized neurotrauma rehabilitation.

Disclosures: **M. Quezada:** None. **K. Cotton:** None. **J.K. Phillips:** None. **J.D. Finan:** None. **C.K. Franz:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.15/A15

Topic: A.04. Transplantation and Regeneration

Support: Halo Award

Title: Establishing a co-culture model of human induced pluripotent stem cell-derived motor neurons and primary stem cell-derived myotubes from patients with cerebral palsy

Authors: L. A. SIBLEY[1], C. K. FRANZ[2,3,4], A. A. DOMENIGHETTI[1,3];
[1]Biologics, Shirley Ryan AbilityLab, Chicago, IL; [2]Biologics, Shirley Ryan Abilitylab, Chicago, IL; [3]Physical Med. and Rehabil., [4]Neurol., Northwestern Univ., Chicago, IL

Abstract: Cerebral palsy (CP) is a non-progressive perinatal brain injury that causes progressive muscle weakness and impaired mobility. The brain injury disrupts the motor control centers and causes a miscommunication between the motor nerve system and skeletal muscles. Treatment therapies for muscle impairment focus on muscle stretching and strengthening, and while these therapies have been used for decades, they are minimally effective in preventing muscle weakness and wasting. Much of the biological research of CP is related to the impact the brain injury has on either the nervous system or skeletal muscle. There is a large gap in knowledge

where these two systems meet at the neuromuscular junction (NMJ) and the miscommunication between organ systems that is occurring in this disorder. Previous research has suggested that the NMJs are structurally dysmorphic in CP, but little is known about why this is or the downstream impact it has on changes in muscle growth patterns. Our goal is to establish a human in vitro assay to study NMJ formation, maintenance and physiology with cells derived from CP patients. To assess formation of NMJs in vitro, we co-cultured human primary stem cell-derived muscle fibers with human pluripotent stem cell (PSC)-derived motor neurons in a dish. The use of all human cell types ensures the patients phenotype is developed in the culture and will show any changes that are specific to CP patients. In our first set of experiments, we used cells from healthy patients to establish our model and to validate the functionality of the co-culture. Our first results showed that after 7 weeks of co-culture, there was evidence of NMJ development in vitro, as evidenced by staining with α-bungarotoxin, a marker for acetylcholine receptors. Field electrophysiology recordings using multi-well multielectrode arrays (Axion Biosystems, USA) also showed that co-cultures were actively producing action potentials, a sign that motor neurons could mature enough to electrically communicate with the muscle fibers. In our ongoing experiments, we are focused on optimizing the protocol for co-culturing motor neurons with muscle fibers and to test our system using cells derived from CP patients. We will be looking at structure, firing patterns, and health of the cells. Illuminating changes in NMJ formation and physiology could open a large field of new possibilities of treatments or drug testing to strengthen the connection between the nerve and muscle systems, and improve motor function in patients with CP.

Disclosures: **L.A. Sibley:** None. **C.K. Franz:** None. **A.A. Domenighetti:** None.

Poster

028. Peripheral Nerve Regeneration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 028.16/A16

Topic: A.04. Transplantation and Regeneration

Title: Improving function after nerve injury via tailored growth factor delivery from mineral coated microparticle

Authors: *D. J. HELLENBRAND, C. HALDEMAN, K. MILLER, M. LOH, N. NOWAK, L. WHEELER, J. GOTCHY, A. DOUCAS, J. FIXEL, C. MOREHOUSE, A. S. HANNA; Neurolgical Surgery, Univ. of Wisconsin, Madison, WI

Abstract: Although autologous nerve grafts are the gold standard for treating large nerve gaps, generally patients only regain a small portion of function in limbs affected by the injury. Current growth factor therapies have shown some promising results, but are often limited with little ability to tailor the growth factor release to a desired timeframe. Ideally, the growth factor will

stay at therapeutic levels long enough for axons to grow the length of the nerve. We hypothesized that mineral coated microparticles (MCMs) will bind, stabilize and release biologically active Glial cell-derived neurotrophic factor (GDNF) and Nerve growth factor (NGF), and growth factor release kinetics will be tailored for the time needed to grow axons the length of graft by adjusting the physicochemical properties of the mineral coatings
To test this hypothesis, mineral coated microparticles loaded with growth factors were incorporated on the distal end of a 10 mm sciatic nerve isograft in male Lewis rats (Fig. 1). The five groups tested were graft with no treatment, MCMs, MCMs + NGF, MCMs + GDNF, MCMs + NGF & GDNF. After grafting, hind limb function was tested until 12 weeks post-operatively by measuring the angle of the ankle at foot lift off while walking down a track. Then rats were testing using electrophysiology, the grafts were harvested, and myelin labeled axons were counted.
At physiological conditions in vitro, the MCMs released NGF and GDNF in a sustained manner for at least 21 days. In vivo, the sustained release of NGF combined with GDNF resulted in significantly more myelinated axons in the graft and distal to the graft, and a significant improvement in hind limb function seven weeks after grafting.

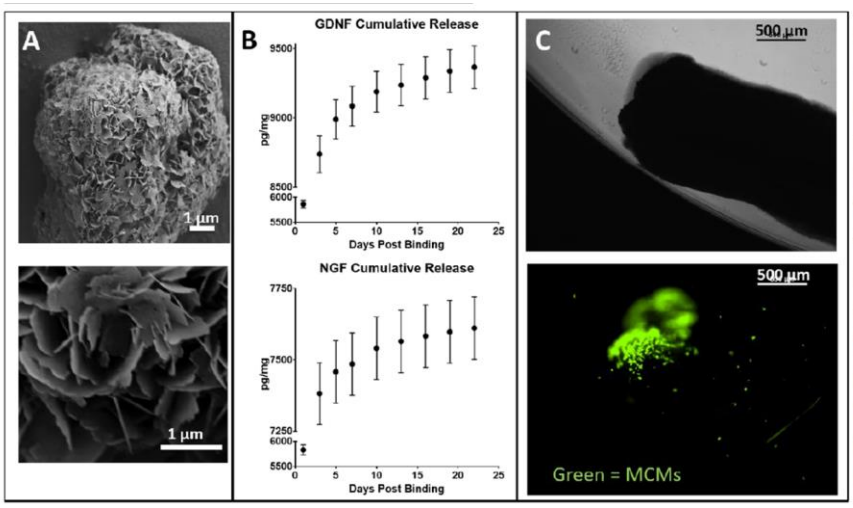

Figure 1. Micrographs of MCMs reveal a continuous nano-porous coating (A). Cumulative release profiles in vitro for both GDNF and NGF show a burst release followed by a sustained release for 22 days (B). The distal end of a nerve graft was dipped in a solution containing MCMS bound to a FITC fluorophore and imaged. Top image brightfield bottom image is the same nerve imaged with FITC filter showing localized MCMs(C).

Disclosures: **D.J. Hellenbrand:** None. **C. Haldeman:** None. **K. Miller:** None. **M. Loh:** None. **N. Nowak:** None. **L. Wheeler:** None. **J. Gotchy:** None. **A. Doucas:** None. **J. Fixel:** None. **C. Morehouse:** None. **A.S. Hanna:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.01/A17

Topic: A.05. Axon and Dendrite Development

Support: This work is supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) (JFW).

Title: Upregulation of antioxidant protein thioredoxin during neuronal differentiation

Authors: M. A. LLANES-CUESTA, *J.-F. WANG;
Pharmacol. and Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: RATIONALE: In the Central Nervous System, neurons differentiate from immature cells, and then the differentiated neurons begin to form synaptic connections and assemble neuronal circuits. During differentiation, immature neurons are highly susceptible to mechanisms promoting cellular death and only functionally appropriate neurons will survive. Neuronal differentiation and survival are critical processes to insure proper neuronal functioning. Impairments in these processes have been linked to the pathogenesis of autism and schizophrenia. Thioredoxin (Trx), as an oxidoreductase, can reverse protein cysteine thiol oxidative modification which plays a major role in cellular redox balance and defense against oxidative stress. Trx can also bind to apoptosis signal-regulating kinase 1 (ASK1) and inhibit ASK1-mediated apoptosis. Trx in a reduced state is maintained by Trx reductase (TrxR). Recent studies have shown that Trx can regulate expression and down-stream signaling of neurotrophic factors, suggesting that Trx may contribute to regulation of neuronal differentiation. **AIM:** The aim of the present study is to understand if Trx plays a role in regulating neuronal differentiation and survival. **METHODS**: Cerebral cortex were dissected from mouse fetus with 17-18 day gestation. Neuronal cells were isolated and cultured in Neurobasal medium with GS21 supplements from 1 to 32 days in vitro (DIV). Trx and TrxR protein levels were measured by western blot analysis. **RESULTS**: Trx and TrxR protein levels were time-dependently increased from 1 DIV to 18 DIV, matching with the period of neuronal differentiation established in scientific literature. **CONCLUSIONS:** Increased Trx and TrxR levels during differentiation suggest that Trx antioxidant system contributes to regulation of neuronal differentiation and survival. Next, we will determine whether suppression of Trx and TrxR inhibit neuronal differentiation and survival.

Disclosures: M.A. Llanes-Cuesta: None. **J. Wang:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.02/A18

Topic: A.05. Axon and Dendrite Development

Support: JSPS KAKENHI 16K14704

Title: Microtubule-associated proteins promote cell protrusion by bundling of actin filaments and microtubules

Authors: *C. DOKI[1], M. KURAGANO[1], K. NISIDA[1], S. SAITO[3], S. KOTANI[4], K. TOKURAKU[2];
[2]Dept. of Applied Sci., [1]Muroran Inst. of Technol., Muroran-Shi, Japan; [3]Muroran Inst. of Technol., Muroran-Shi, Japan; [4]Kanagawa Univ., Kanagawa-Shi, Japan

Abstract: Microtubule-associated proteins (MAPs) have been well known as the proteins that bind to microtubules (MTs) and regulate MT-dependent cellular functions. Since major mammalian MAPs, specifically MAP2, MAP4, and tau, exhibit high structural similarity, these MAPs have formed the MAP2/MAP4/tau superfamily. We recently reported that a part of the Pro-rich region in the microtubule-binding domain (MBD) of MAP4 binds to actin filament (F-actin) (Matsushima et al., 2012). However, physiological roles of this interaction remain unknown. In this study, we tried to elucidate the physiological function of actin-binding activity of MAPs. First, we examined the behavior of MBD fragments of MAP4, MAP2, and tau coexisting with F-actin and MTs using the flow chamber consisted of the cover and slide glass. When the MBD fragments preincubated with MTs were mixed with F-actin, all MAPs failed to form hybrid bundles consisting of F-actin and MTs. On the other hand, when the MAPs preincubated with F-actin were mixed with MTs, not tau but MAP2 and MAP4 formed the hybrid bundles. MAP2 showed significantly higher hybrid bundle-forming activity than that of MAP4. Then, to examine whether MT assembly promoting activities of MAPs affect by binding to F-actin, we observed MT formation with or without F-actin by fluorescence microscopy. The observation showed that MAP2 and MAP4 were significantly promoted MT assembly in the presence of F-actin and formed hybrid bundles. Although tau also induced some MTs,the bundles were smaller than the cases of MAP2 and MAP4. These results suggest that MT assembly-promoting activity of MAP2 and MAP4 was enhanced by coexistence of F-actin. Next, we investigated the morphological effect on cell shape by over expression of EGFP-MAP4 using neuroblastoma-glioma hybrid cells, NG108-15 cells. EGFP-MAP4 were colocalized with MT and F-actin in protrusions cells. The number of protrusions and the maximum length of protrusion significantly increased by overexpression of EGFP-MAP4. Meanwhile, the average protrusion length was not changed. These results suggest that MAP4 mediated interaction between F-actin and MT might be involved in the formation and stabilization of protrusions in the NG108-15 cells. In this study, we demonstrated that MBD of MAP2 and MAP4 is required for F-actin-dependent MT assembly-promoting activity in *vitro*, and that MAP4 promoted formation of cell protrusion including MT and F-actin in NG108-15 cells. These results imply that the F-actin-dependent MT assembly-promoting activity and stabilization of F-actin-MT bundle by MAPs might be involved in formation and maintaining the proper structure of neural tissue in vivo.

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Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.03/A19

Topic: A.05. Axon and Dendrite Development

Support: Smoking Research Foundation

Title: Nicotine inhibits neurite outgrowth via α_7 and/or $\alpha_3\beta_4$ nicotinic acetylcholine receptors in PC_{12} cells

Authors: *H. KAWASAKI[1], S. TAKATORI[1], Y. KONDO[1], Y. YURITA[1], H. SAGARA[1], H. HINO[2], F. TAKAYAMA[2];
[1]Dept. of Clin. Pharmacy, Col. of Pharmaceut. Sciences, Matsuyama Univ., Matsuyama, Ehime, Japan; [2]Grad. Sch. of Medicine, Dent. and Pharmaceut. Sciences, Okayama Univ., Okayama, Okayama, Japan

Abstract: ***Background:*** Nicotine has been shown to re-innervate perivascular sympathetic adrenergic nerves lesioned by topically applied phenol in the rat mesenteric artery *in vivo*, and increase levels of nerve growth factor (NGF) contents and the expression of NGF receptor TrkA in superior cervical ganglia (SCG) (Takatori S *et al*., Eur J Pharmacol, 2015.). Furthermore, we reported that low doses of nicotine facilitates neurite outgrowth of primary cultured SCG cells via activation of α7 nicotinic acetylcholine receptors (nAChR) *in vitro*, while high dose nicotine inhibited neurite out growth, suggesting that nicotine has dual effect of facilitation and inhibition on the neurite outgrowth in SCG cells (Neuroscience Meeting, 2017). The aim of this study is to examine effects of nicotine on neurite outgrowth in PC12 cells *in vitro*.
Methods: PC12 cells were cultured in RPMI1640 medium supplemented with different concentrations of nicotine (1 μM-100 mM) for 7 days. Numbers of neurite outgrowth and branches and length of the neurite from cell body were measured during the experiment. A non-selective nAChR antagonist hexamethonium (100 μM, Hex), a selective α7 nAChR antagonist α-bungarotoxin (100 nM, Bgtx) or a selective α3β4 nAChR antagonist SR16584 (100 μM) was co-incubated with 100 mM nicotine for 7 days.
Results: Nicotine at low concentrations of 1-10 μM concentration-dependently increased only length of the neurite from PC12 cell body, which was inhibited by Hex and Bgtx. However, high concentrations of 1-100 mM nicotine caused a concentration-dependent decrease in numbers and branches of neurite outgrowth and length of the neurite. The inhibitory effect of 100 mM nicotine on length of the neurite was cancelled by the treatment of Hex, Bgtx or SR16584.
Conclusions: These results suggest that nicotine has dual effects of facilitation and inhibition on length of the neurite in PC12 cells, and inhibitory effect induced by high concentration of

nicotine is mediated by α7 and/or α3β4 nAChR. (This study was supported by Smoking Research Foundation).

Disclosures: **H. Kawasaki:** None. **S. Takatori:** None. **Y. Kondo:** None. **Y. Yurita:** None. **H. Sagara:** None. **H. Hino:** None. **F. Takayama:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.04/A20

Topic: A.05. Axon and Dendrite Development

Support: Japan MEXT 26640024
Toshimi Otsuka Scholarship

Title: Promotion of axon and dendrite formation by serotonin 4 receptor through collapsing response mediator protein-2

Authors: L. AGRAWAL, *T. SHIGA;
Univ. of Tsukuba, Tsukuba, Japan

Abstract: Serotonin (5-hydroxytryptamine, 5-HT) is a monoaminergic neurotransmitter involved in the early brain development via multiple molecular mechanisms. In the brain availability of 5-HT pool is controlled via 5-HT receptors and transporters. The 5-HT4R subfamily is linked to Gs proteins, which is involved in the early brain development. Our group has previously reported the role of 5-HT4R in the dendrite formation of hippocampus neurons. However, the role of 5-HT4R in hippocampal development is not completely understood. Therefore, in the present study we extensively examined the role of 5-HT4R during early brain development in dendrite and axon formation of hippocampal neurons. Results showed that activation of 5-HT4R through agonist RS67333 significantly increased the axonal length, diameter and branching along with notable improvement of total length of dendrites, number of primary dendrites and their branching. In contrast, RS67333-induced growth of axon and dendrite was neutralized by the concomitant treatment with a 5-HT4R antagonist GR125487, confirming the specific role of the 5-HT4R in neurites formation. Furthermore, treatment with RS67333 increased the mRNA expression of neurotrophic factors BDNF, NT-3, NGF together with collapsin response mediator protein-2 (CRMP2) and non-phosphorylated CRMP2, suggesting the possible downstream singling via 5-HT4R. In summary, the current study enriches the understanding and key role of 5-HT4R in early embryonic development of hippocampal neurons.

Disclosures: **L. Agrawal:** None. **T. Shiga:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.05/A21

Topic: A.05. Axon and Dendrite Development

Support: JMU Department of Biology
JMU Light Microscopy and Imaging Facility
JMU College of Science and Mathematics Summer Faculty Assistance Grant
4-VA, a collaborative partnership for advancing the commonwealth of Virginia
JMU College of Science and Mathematics Farrell scholarship
JMU College of Science and Mathematics Butler scholarship
JMU College of Science and Mathematics Jeffrey E. Tickle '90 Family Endowment scholarship

Title: Integrin beta 3 regulates a tangential, orderly gradient of dendritic arborization in layer II/III pyramidal neurons

Authors: Z. L. HOLLEY, K. M. BLAND, A. J. LOPUCH, M. I. SONG, B. D. SWINEHART, E. L. WIDENER, Z. O. CASEY, C. J. HANDWERK, *G. S. VIDAL;
James Madison Univ., Harrisonburg, VA

Abstract: Integrin subunits have been implicated in axonal and dendritic outgrowth, as well as dendritic spine plasticity. In particular, a strong positive association has been found between mutations in integrin beta 3 (Itgb3) and autism spectrum disorder, but little is known about the role of Itgb3 on neuronal structure and function in vivo. Many forms of autism spectrum disorder are thought to arise from dysfunctional dendritic arborization and synaptic pruning, and global knockout of Itgb3 in mice leads to autistic-like behaviors. Previously, we have shown that Itgb3 is required for normal dendritic arborization in layer II/III pyramidal neurons of mouse neocortex in a cell-specific manner. Furthermore, it is known that dendritic morphology of mouse layer II/III excitatory pyramidal neurons across much of the tangential plane of the cerebral cortex is partly shaped by a developmental, orderly gradient spanning several functional regions. Therefore, here, we hypothesized that the orderly tangential gradient of dendritic arborization is abrogated by the cell-specific loss of Itgb3 in vivo. This was achieved by causing Itgb3 loss of function through Cre-lox-mediated excision of Itgb3 in a subset of layer II/III cortical neurons. Layer II/III cortical neurons were targeted for excision via in utero electroporation of GFP/Cre DNA constructs to the ventricular zone of developing telencephalon of mice in which exon 1 of Itgb3 is flanked by loxP sites. Cortical positioning on the tangential plane of the cortex as well as dendritic morphological features of targeted neurons in juvenile mice (P23) were analyzed. Male and female mice were used for the study and analysis was done blind to genotype. Results point

to the loss of an orderly gradient of dendritic arborization in mutant neurons, when compared to C57BL6/J controls. Thus, integrin beta 3 appears to regulate a tangential, orderly gradient of dendritic arborization of layer II/III pyramidal neurons in the developing neocortex.

Disclosures: Z.L. Holley: None. **K.M. Bland:** None. **A.J. Lopuch:** None. **M.I. Song:** None. **B.D. Swinehart:** None. **E.L. Widener:** None. **Z.O. Casey:** None. **C.J. Handwerk:** None. **G.S. Vidal:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.06/A22

Topic: A.05. Axon and Dendrite Development

Support: Gates Center for Regenerative Medicine - CU Denver Anschutz Medical Campus
Department of Pediatrics - CU Denver Anschutz Medical Campus
Children's Hospital of Colorado Research Institute
Boettcher Foundation
Linda Crnic Institute for Down Syndrome
CCTSI

Title: Csmd2 is required in neuronal maturation and Reelin-mediated structural plasticity

Authors: *M. A. GUTIERREZ, B. E. DWYER, S. J. FRANCO;
Univ. of Colorado Denver, Anschutz Med., Denver, CO

Abstract: Reelin is a secreted glycoprotein that regulates development of the cerebral cortex. By initiating a signaling cascade through the downstream adaptor protein, Dab1, Reelin has been studied as a migration guidance signal for immature neurons in the developing brain. Reelin loss-of-function results in disorganized neuronal layering, significant reduction of dendritic arbor complexity, and deficits in dendritic spine development. While the mechanisms through which Reelin controls neuronal migration have been extensively investigated over the years, the mechanisms that regulate dendrite and spine development downstream of Reelin and Dab1 have yet to be fully elucidated. Here, we have identified a novel interaction between Dab1 and Csmd2, a single-pass transmembrane protein of previously unknown function. We demonstrate that Csmd2 is expressed in the cerebral cortex and that Dab1 binds to the FENPMY motif in the cytoplasmic tail of Csmd2. Additionally, we show that Csmd2 is enriched in excitatory and inhibitory neurons in the forebrain. Interestingly, we find that Csmd2 interacts with several synaptic scaffolding proteins and localizes to dendritic spines through a PDZ motif-mediated interaction with PSD-95. Knockdown of Csmd2 mRNA in cultured hippocampal neurons results in reduced complexity of dendritic arbors and deficits in dendritic spine density. Knockdown of

Csmd2 mRNA expression in developing neurons results in reduced filopodia density, whereas knockdown of Csmd2 mRNA in mature neurons causes significant reductions in dendrite complexity and dendritic spine density. Finally, knockdown of Csmd2 mRNA or expression of a truncated form of Csmd2 causes a significant reduction in dendritic spine density and dendrite complexity that is unable to be rescued by Reelin. Together, these data indicate a role for Csmd2 in dendritogenesis and dendritic spine development and stability. This suggests that Csmd2 may be a critical factor downstream of Reelin/Dab1 during neuronal maturation, which may account for its association with certain neuropsychiatric disorders.

Disclosures: M.A. Gutierrez: None. **B.E. Dwyer:** None. **S.J. Franco:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.07/A23

Topic: A.05. Axon and Dendrite Development

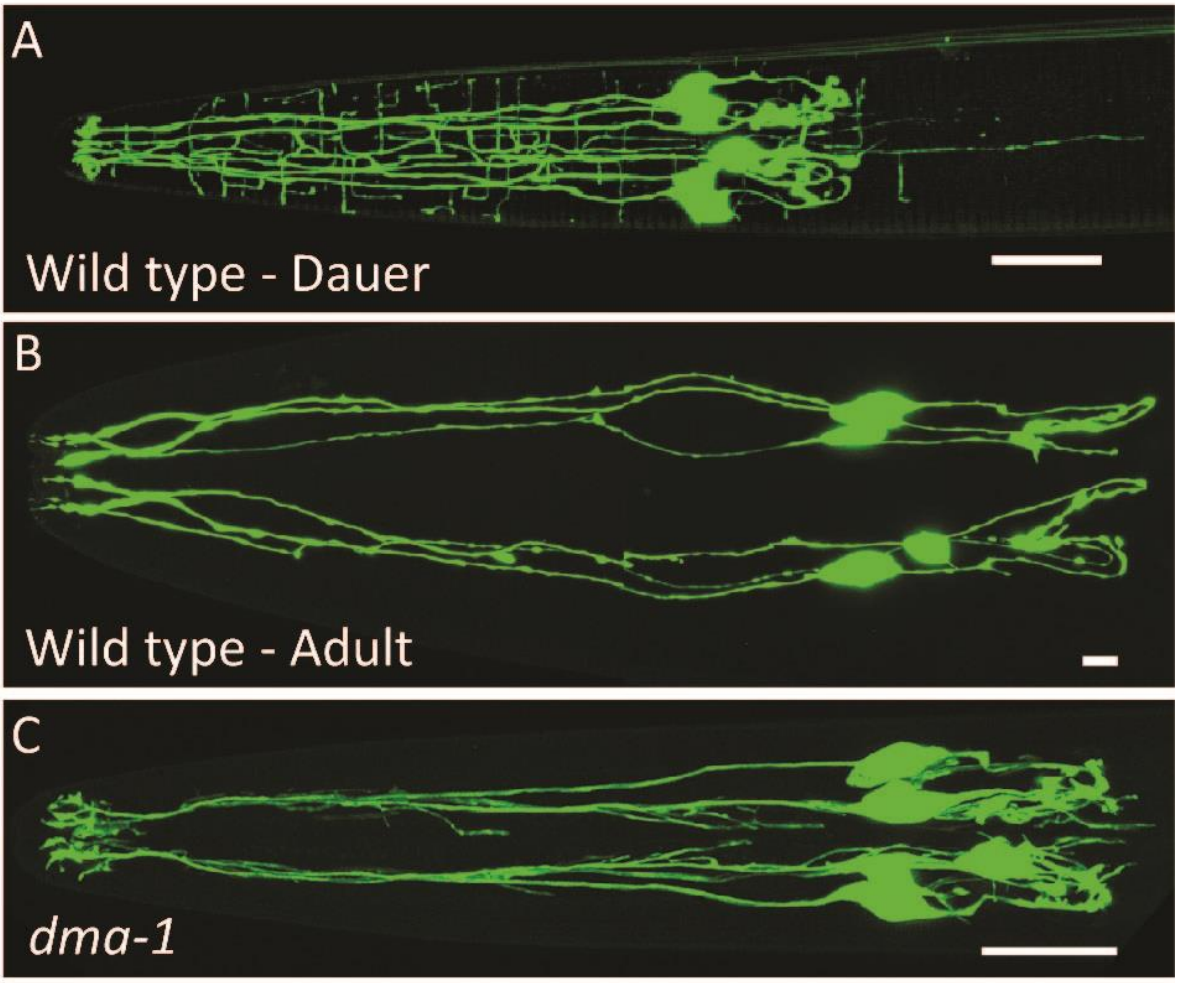
Support: NSF Grant 1559908
NSF Grant 1559929
NIH Grant R01GM111566

Title: Stress-induced dendrite arborization in *C. elegans*

Authors: *R. ANDROWSKI, J. GOELZER, A. HOFER, C. SMITH, N. SCHROEDER;
The Univ. of Illinois, Urbana, IL

Abstract: Dendrite morphology plays a key role in proper neural signaling. Stress can impact dendrite arborization; however, little is known about the underlying molecular mechanisms that control this process. We use the nematode *C. elegans* as an *in vivo* model to investigate stress-induced neural plasticity. When grown under conditions of low food and high population density, *C. elegans* enters dauer diapause. When dauers are returned to favorable conditions they continue development. Dauers undergo widespread arborization of the 4 quadrant inner labial 2 (IL2) neurons. The typically unbranched IL2 dendrite arborizes extensively when the animal enters dauer, increasing its total branch length 3-fold (Figure A). Upon dauer exit the neuron resorbs its arbors, returning to an unbranched morphology (Figure B). We performed a forward genetic screen to identify regulators of IL2 morphology. Through this screen, I generated a new allele of the gene *dma-1* (Figure C). We found that a membrane-bound complex centered on DMA-1 was repurposed during dauer to enable IL2 branching. DMA-1 had previously been implicated in arborization of the branched neurons, the PVDs. Using a DMA-1 translational reporter, we show that DMA-1 is expressed in the IL2s during dauer and localizes to the IL2 dendrites. Several extracellular binding partners of DMA-1 have been identified including the

L1CAM homolog, SAX-7. Separately DMA-1 functions intracellularly in the PVDs to initiate actin polymerization. Through an analysis of DMA-1 associated molecules, we determined that the DMA-1 extracellular and intracellular binding partners are necessary for IL2 arborization. Next, we looked for regulators of IL2 arborization among stress-related pathways. The FOXO transcription factor DAF-16 is part of the Insulin signaling pathway and is necessary for dauer formation. We found that DAF-16 is required for stress-induced dendrite morphology but not arborization of unstressed neurons.



Disclosures: R. Androwski: None. **J. Goelzer:** None. **A. Hofer:** None. **C. Smith:** None. **N. Schroeder:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.08/A24

Topic: A.05. Axon and Dendrite Development

Support: NSF IOS-1753730
NIH NIGMS P20GM103423

Title: Semaphorin 1a and deafferentation influence the structure and function of the auditory system of the cricket Gryllus bimaculatus

Authors: *H. W. HORCH[1], J. D. MOYNIHAN[1], S. G. BRILL-WEIL[1], P. S. DICKINSON[2]; [1]Bowdoin Col., Brunswick, ME; [2]Bowdoin Coll, Brunswick, ME

Abstract: Although large scale structural plasticity in adult nervous systems is rare, some organisms maintain the ability to make compensatory structural changes into adulthood, offering insights into the mechanisms underlying structural plasticity in mature nervous systems. These organisms can also help us understand the ramifications of structural plasticity for nervous system function and behavior in the adult. The cricket, *Gryllus bimaculatus*, exhibits unique and robust deafferentation-induced anatomical reorganization in the auditory system. In the cricket, two auditory organs on each forelimb send auditory afferents via nerve 5 to the prothoracic ganglion (PTG), where auditory afferents synapse onto mirror-image pairs of auditory neurons, notably Ascending Neurons 1 and 2 (AN1 and AN2). These cells receive auditory input only from the ipsilateral ear because auditory system dendritic and axonal arbors are restricted by the midline. Upon unilateral loss of an ear, the deafferented ipsilateral AN1 and 2 dendrites sprout across the midline of the PTG, forming functional synapses with contralateral auditory afferents. Here we examined two aspects of these changes. First, to assess the physiological and anatomical consequences of this deafferentation-induced reorganization, we recorded AN responses to sound using a suction electrode on the surface of the brain followed by iontophoresis of dye into AN axons; this backfills the AN dendrites in the PTG and allows us to correlate physiological and anatomical data from individual animals. Surprisingly, unilateral deafferentation caused rapid and permanent synaptic reorganization of the auditory system within the brain, which was independent of the structural reorganization that occurs in the PTG. Second, we asked whether altering the expression of specific proteins could predictably manipulate AN form and function. For example, the expression of *sema1a* mRNA declines rapidly after deafferentation. To test whether this correlative change in expression causes changes to AN morphology, we knocked down *sema1a* mRNA in adult intact animals by peripheral injection of double-stranded RNA. We assessed the anatomy and sound-driven physiological responses of the AN neurons using surface electrodes on the brain followed by iontophoresis. In response to *sema1a* dsRNA

knockdown in intact animals, AN dendritic arbors changed shape, increased in complexity, and sprouted dendrites across the midline. We will begin to correlate compensatory anatomical changes with physiological responses as we gain insight into the molecular mechanisms that are involved in this adult compensatory reorganization.

Disclosures: H.W. Horch: None. **J.D. Moynihan:** None. **S.G. Brill-Weil:** None. **P.S. Dickinson:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.09/A25

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01NS104078
NIH Grant R15MH101703

Title: LAMTOR1/p18 controls dendritic trafficking and positioning of lysosomes by regulating TRPML1-mediated lysosomal calcium release

Authors: *J. SUN, Y. LIU, X. HAO, W. LIN, E. CHIANG, B. DIEP, M. BAUDRY, X. BI; Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Acylation of p18, also known as LAMTOR1, is essential for anchoring to endosomal/lysosomal membranes the Ragulator complex, which has been shown to a play critical role in activating the mechanistic target of rapamycin complex 1 (mTORC1), an important component of synaptic plasticity and learning and memory. We have previously reported that p18 is essential for lysosomal localization of Ragulator and RagGTPases in hippocampal neurons, and that UBE3A-mediated p18 ubiquitination and degradation regulate mTORC1 activity. Recent studies have shown that the Ragulator complex inhibits lysosome centrifugal trafficking in an mTORC1-independent manner in cell lines. However, little information is available regarding the role of p18 in lysosomal trafficking in neuronal dendrites. Here we show that p18 knockdown (KD) in primary neurons increases lysosome motility in proximal dendrites. Live-cell imaging of lysosomes stained with LysoTracker showed that p18 KD markedly decreased the proportion of stationary lysosomes and increased that of moving lysosomes; both anterograde and retrograde motility of lysosomes in dendrites was enhanced. Furthermore, both the total distance and average velocity of mobile lysosomes increased upon p18 KD. In addition, quantitative analysis of lysosomes stained with LAMP2 revealed a significant increase in the number of lysosomes in proximal dendrites. Of note, inhibition of mTORC1 activity by rapamycin or Torin1 had no effect on lysosome distribution in dendrites, suggesting that lysosome positioning in dendrites depends on p18 but not on mTORC1. We

identify transient receptor potential mucolipin 1 (TRPML1) as a novel interacting protein for p18. TRPML1-mediated lysosomal Ca^{2+} release, measured using a genetically encoded Ca^{2+} indicator attached directly to TRPML1, was significantly increased after p18 KD, suggesting that p18 exerts a negative regulatory effect on TRPML1. Incubation with the TRPML1 antagonist MLSI1 fully restored lysosomal trafficking and positioning in dendrites of p18 KD neurons, supporting a functional interaction between p18 and TRPML1. Interestingly, inhibition of the "retrograde" motor dynein with Ciliobrevin D in p18 KD neurons not only reduced the retrograde movement but also the anterograde movement of lysosomes, suggesting that dynein participates in bidirectional lysosomal trafficking in dendrites. Overall our results suggest that p18/TRPML1 interaction is crucial for controlling dendritic trafficking of lysosomes, presumably by controlling the motor protein dynein.

Disclosures: J. Sun: None. **Y. Liu:** None. **X. Hao:** None. **W. Lin:** None. **E. Chiang:** None. **B. Diep:** None. **M. Baudry:** None. **X. Bi:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.10/A26

Topic: A.05. Axon and Dendrite Development

Title: Celsr2 regulates the development of mouse retianl horizontal cells via Wnt5b mediated Wnt PCP signaling

Authors: *J. ZHANG, Y. QU;
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Abstract: Celsr2, a seven-pass transmembrane cadherin, is one of the core planar cell polarity (PCP) proteins. Other members of the PCP genes such as *Fzd3* and *Celsr3* were shown previously to be involved in retina development, but little is known about *Celsr2*. Here, we investigated the role of Celsr2 in the development of mouse retinal horizontal cells. *Celsr2* is expressed in retinal horizontal cells, ganglion cells and Müller cells. Abnormal neurite sprouting of horizontal cells was observed in *Celsr2* knockout mice. Retinal function is decreased in *Celsr2* knockout mice based on ERG recording. JNK is up regulated in *Celsr2* knockout mice, indicating that the non-canonical Wnt-PCP-JNK pathway is involved. In order to find the upstream of *Celsr2*, we generated double heterozygous models of *Wnt5a+Celsr2* and *Wnt5b+Celsr2*. Retinal horizontal cell sprouting was only observed in the double heterozygote of *Wnt5b* and *Celsr2*, suggesting *Wnt5b* may be the upstream of *Celsr2*.Then we studied *Wnt5b* knockout mice, similar neurite sprouting
phenotype was found in retinal horizontal cells. Fzd family is known as classical Wnt receptors, here we established a *Fzd3+Wnt5b* double heterozygous mouse model and found similar

phenotype in horizontal cells. These results suggest that Celsr2 regulates neurite outgrowth of retinal horizontal cells via Wnt5b mediated Wnt-PCP signaling in mouse retinal development. These results also indicate Fzd3 may combine with Celsr2 as the *Wnt5b* receptor to regulate the development of mouse retina.

Key words: : *Celsr2,* retinal horizontal cells, *Fzd3, Wnt5b, neurite sprouting*

Disclosures: J. Zhang: None. **Y. Qu:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.11/A27

Topic: A.05. Axon and Dendrite Development

Support: SFB 2016 Project A06

Title: Deciphering ste20-like kinase signaling in neurons

Authors: *A. QUATRACCIONI[1], B. ROBENS[1], S. AHMADI[2], D. WINTER[2], M. GRAHAM[3], A. WAARDENBERG[4], A. J. BECKER[1], S. SCHOCH[1];
[1]Inst. of Neuropathology, Univ. of Bonn Med. Ctr., Bonn, Germany; [2]Inst. of Biochem. and Mol. Biol., Univ. of Bonn, Bonn, Germany; [3]Synapse Proteomics, [4]Bioinformatics Unit, Children's Med. Res. Inst., Westmead, Australia

Abstract: The Ste20-like kinase (SLK) fulfills important and diverse functions in non-neuronal cells by influencing cellular processes such as apoptosis, cell cycle progression, cytoskeletal dynamics, and cell migration. Surprisingly, almost nothing is known about the functional relevance of SLK in neurons, even though the kinase is expressed in neurons throughout development and adulthood and the brain fails to develop normally in constitutive knockout mice. Furthermore, SLK was recently reported as candidate gene for intellectual disability pointing to an important role in neurons. In non-neuronal cells, several molecular pathways involving SLK have been described, for example integrin signaling to the cytoskeleton or regulation of kinases in the cell cycle. However, the mechanism underlying SLK action in neurons is currently unknown. To gain first insights into SLK's mode of action in neurons, we are investigating interaction partners and phosphorylation substrates of SLK by three complementary strategies. In a whole-cell phosphoproteomics approach, SLK is either overexpressed or knocked down by lentiviral transduction and phosphorylated proteins are analyzed by mass spectrometry. The second strategy focuses on the identification of transient cellular interaction partners through proximity-dependent biotinylation. Viral transduction of cultured cortical neurons leads to the expression of a SLK-biotin ligase fusion protein that biotinylates proteins in close proximity to SLK. Subsequent mass spectrometric analysis of the

isolated biotinylated proteins specifically identifies proteins that are in the vicinity of SLK in neuronal cells. A traditional co-immunoprecipitation analysis complements the search for interacting proteins with direct binding partners of SLK. The combination of these approaches allows us to identify proteins that act up- or downstream of SLK. In the long term, these first insights into the molecular mechanism of SLK will lead to a better understanding of SLK's function in neuronal development and clarify how the kinase influences morphological and functional properties of neurons.

Disclosures: A. Quatraccioni: None. **B. Robens:** None. **S. Ahmadi:** None. **D. Winter:** None. **M. Graham:** None. **A. Waardenberg:** None. **A.J. Becker:** None. **S. Schoch:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.12/A28

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant 5R01MH107305-03
NIH Grant 5T32GM008490-25

Title: Control of dendritic arborization in *Drosophila* by an intellectual disability-associated RNA-binding protein and planar cell polarity components

Authors: *E. B. CORGIAT, III[1], J. C. ROUNDS[2], S. M. LIST[2], P. CHEN[1], A. H. CORBETT[3], K. H. MOBERG[1];
[1]Cell. Biol., [3]Biol., [2]Emory Univ., Atlanta, GA

Abstract: The human *ZC3H14* gene encodes a ubiquitously expressed zinc-finger RNA-binding protein that is lost or altered in an inherited form of non-syndromic, recessive intellectual disability. To gain insight into neurological defects in patients lacking ZC3H14, we previously developed a *Drosophila melanogaster* model of ZC3H14 loss by deleting it’s fly ortholog, Nab2. Nab2-deficient animals have defects in locomotion and olfactory memory that are rescued by Gal4-UAS mediated expression of fly Nab2 or human ZC3H14 solely in fly neurons. This rescue implies a high degree of conservation between molecular and cellular roles of ZC3H14 and Nab2 in neurons. Nab2 loss leads to axon projection defects in a region of the fly brain termed the mushroom bodies (MB), a twin neuropil structure composed of fasiculated axons derived from Kenyon neurons. In prior publication, we showed that Nab2 controls MB axon development in a complex with the fly Fragile X homolog, dFmrp. Here, we reveal that Nab2 also has an unappreciated role in limiting dendrite development among body-wall sensory neurons. RNAi of dNab2 within larval class IV dorsal dendritic arborization C (ddaC) neurons significantly increases dendritic complexity by multiple measures (i.e. Sholl analysis, total arbor length, and

maximum branch order), and excess Nab2 has the inverse effect. ddaC dendrite defects are also evident in Nab2 null animals, arguing that Nab2 regulates mRNA(s) involved in dendrite development. The genetic screen that identified dFmrp as a Nab2 interactor also identified multiple members of the non-canonical Wnt, Planar Cell Polarity pathway (PCP) as Nab2 interacting genes. We are testing the possibility that PCP contributes to Nab2 dendrite arbor phenotypes and have confirmed that RNAi of multiple PCP components yield defects in the ddaC arbor, confirming published work that ddaC neurons use the PCP pathway to control dendrite arborization. We are currently testing whether Nab2 alters levels of PCP proteins in vivo, and whether the ddaC dendrite defects in Nab2 null/RNAi animals can be rescued by alleles that reduce PCP activity. Based on our published data showing ZC3H14 localizes to RNP-like structures within the dendritic shaft and spines of cultured mouse hippocampal neurons, we theorize that Nab2 and ZC3H14 may have conserved role in regulating RNAs involved in dendrite morphology.

Disclosures: **E.B. Corgiat:** None. **J.C. Rounds:** None. **S.M. List:** None. **P. Chen:** None. **A.H. Corbett:** None. **K.H. Moberg:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.13/A29

Topic: A.05. Axon and Dendrite Development

Support: University of Wisconsin-Milwaukee Research Growth Initiative

Title: Developmental changes in the intrinsic excitability and morphology of ventral hippocampal CA1 neurons

Authors: *V. L. EHLERS[1], H. YOUSUF[1], M. D. LINSKE[1], J. R. MOYER, Jr.[1,2];
[1]Univ. of Wisconsin Milwaukee Dept. of Psychology, Milwaukee, WI; [2]Univ. of Wisconsin Milwaukee Dept. of Biol. Sciences, Milwaukee, WI, United States, Milwaukee, WI

Abstract: The ventral hippocampus forms reciprocal connections with the amygdala (Cenquizca & Swanson, 2007), making it well-suited to contribute to emotional learning, including associative fear learning. However, the role of early postnatal development in forming the necessary physiological mechanisms and morphological connections in ventral hippocampus that support learning in adulthood remains unknown. Development in other brain regions critical for fear learning (e.g. amygdala) is characterized by dynamic neuronal physiology and maturation of dendritic connections. Developing principal neurons in basolateral amygdala (BLA) display more hyperpolarized action potential (AP) thresholds and narrower APs, as well as reduced medium afterhyperpolarizations (mAHPs; Ehrlich et al., 2012). Morphologically, developing

BLA neurons display increased dendritic arborization and complexity (Ryan et al., 2016). These changes in excitability and morphology in developing BLA neurons likely contribute to the pattern of responding to emotionally-salient stimuli in adulthood. Although ventral hippocampus has a known role in fear conditioning and likely routes associative fear information to BLA, developmental changes in ventral hippocampal neuron physiology and morphology have not been characterized. By combining *in vitro* whole-cell recordings from ventral hippocampal CA1 neurons with confocal microscopy and neuronal reconstructions, our lab is exploring how early postnatal development contributes to the maturation of intrinsic excitability and dendritic arborization in ventral CA1. Recordings of ventral CA1 neurons from male F344 rats younger than postnatal day 30 (P30) suggest that development is associated with distinct changes in AP properties and intrinsic excitability. As ventral CA1 neurons mature, AP half-width and AP threshold decrease, while AP amplitude increases ($p < .01$). Additionally, intrinsic neuronal excitability is reduced, as both the slow afterhyperpolarization (sAHP) and mAHP become larger during development ($p < .05$). Reconstructions of these neurons also reveal several developmental changes in ventral CA1 neuronal morphology, including an increase in total dendritic branch length and branch number ($p < .05$), suggesting dendritic arborization becomes more complex during this period of development. These data demonstrate that ventral hippocampal CA1 neurons are physiologically and morphologically dynamic during development, which likely contributes to the emergence of emotional processing.

Disclosures: V.L. Ehlers: None. **H. Yousuf:** None. **M.D. Linske:** None. **J.R. Moyer:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.14/A30

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant F31NS100370
NIH Grant R01NS096672

Title: The function of a glycosylation enzyme (AMAN-2/Man2a) in somatosensory dendrite patterning

Authors: *M. RAHMAN, C. A. DIAZ-BALZAC, H. E. BÜLOW;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Dendrite development is essential for the transmission and processing of sensory stimuli. Abnormalities in dendrite morphology have been found in several neurological disorders. We use the *C. elegans* PVD neurons, which have complex menorah-like dendritic arbors, as a model to study the genes involved in dendritogenesis. Studies have shown that a

conserved cell-adhesion complex, comprised of MNR-1/Menorin and SAX-7/L1CAM, acts from the skin to regulate PVD dendrite branching through the transmembrane receptor, DMA-1/LRR-TM. Recently, we determined that *Leukocyte Cell-Derived Chemotaxin 2*, or *lect-2/Chondromodulin II*, also functions to pattern PVD dendrites. In order to identify genetically interacting factors of LECT-2/ChM-II, we performed a forward genetic screen to isolate modifiers of a *lect-2/ChM-II* hypomorphic allele. We determined that mutations in *aman-2/Golgi alpha-mannosidase II*, an enzyme that is required for the formation of complex N-glycans, enhances the severity of the *lect-2/ChM-II* and *mnr-1/Menorin* hypomorphic phenotypes, but looks wildtype on its own. AMAN-2 acts cell-autonomously to rescue defects in PVD, suggesting that N-glycosylation of a 'menorin' complex component in PVD itself, such as DMA-1/LRR-TM, may be essential. To test this hypothesis, we first performed Western blot analysis after treating proteins with a reagent that cleaves all N-glycans. We established that DMA-1/LRR-TM is glycosylated *in vivo*, and that the glycan profile of DMA-1 is altered in animals lacking AMAN-2. We aim to test our candidate by selectively mutating its N-glycosylation sites. We will further characterize the role of *aman-2/GM-II* in the binding of the 'menorin complex' in future pull down assays to gain a fuller understanding of this novel factor and its *in vivo* role in dendrite development.

Disclosures: M. Rahman: None. **C.A. Diaz-Balzac:** None. **H.E. Bülow:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.15/A31

Topic: A.05. Axon and Dendrite Development

Support: JSPS KAKENHI 18J10215

Title: Bmp signaling via LIMK regulates developmental remodeling of mitral cells dendrites

Authors: *S. AIHARA, T. IMAI;
Grad. Sch. of Med. Sci., Kyushu Univ., Fukuoka, Japan

Abstract: In the mammalian brain, neurons initially form excessive neurites, and then remodel their morphology during early postnatal development. Mitral cells in the olfactory bulb initially extend multiple dendrites, but just one of them is strengthened to form a discrete primary dendrite and the supernumerary ones are pruned during development. We recently found that neuronal activity is essential for the dendrite pruning of mitral cells (Fujimoto, Leiwe et al., *bioRxiv* 2019). However, it has remained unknown how the dendrite stability is controlled intracellularly. It has also been unknown whether cell surface proteins also account for this process. Using *in utero* electroporation, we performed CRISPR/Cas9-based knockout screening

for genes that regulate dendrite remodeling in mitral cells. We found that the mitral cell-specific knockout of BMP receptor type 2 (BMPR2) results in the formation of multiple primary dendrites. Rescue experiments with deletion mutants revealed that the C-terminal domain critical for LIMK regulation, rather than the kinase domain necessary for the canonical Smad pathway, is essential for normal dendrite remodeling. Active LIMK is known to inhibit cofilin by phosphorylation and thereby stabilize actin cytoskeleton. Supporting this notion, LIMK1 overexpression also resulted in the formation of multiple primary dendrites; however, this phenotype was rescued by the overexpression of BMPR2. Thus, BMPR2 (most likely, the ligand-unbound form) is a negative regulator for LIMK and actin polymerization, and thereby facilitates dendrite pruning. On the other hand, overexpression of BMP4 stabilized multiple primary dendrites, suggesting that ligand-bound BMPR2 activates LIMK and stabilizes the dendrites. To examine possible crosstalk of BMPR2-LIMK pathway with neuronal activity, we tested possible roles of activity-dependent Rho-family GTPases and kinase/phosphatases. We found that overexpression of Rac1 produces multiple primary dendrites, while this phenotype was rescued by LIMK1 knockout, suggesting that Rac1 signaling also stabilizes the dendrites via LIMK. We also found that overexpression of a cofilin phosphatase, Slingshot, rescued the LIMK1 overexpression phenotype (multiple primary dendrites). These results suggest that the regulation of actin cytoskeleton by BMPR2, Rac1, and Slingshot, is critical for the stabilization vs. pruning of mitral cell dendrites.

Disclosures: S. Aihara: None. **T. Imai:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.16/A32

Topic: A.05. Axon and Dendrite Development

Support: Fondation Léon Fredericq Grant
Rotary Clubs Grant

Title: Unravelling the role of Cdk7 in postmitotic cortical neurons

Authors: *S. VERTENEUIL[1], Q. MARLIER[1], D. SANTAMARÍA[3], M. BARBACID[4], L. NGUYEN[2], R. VANDENBOSCH[1], B. MALGRANGE[1];
[1]Developmental Neurobio. Unit, [2]Mol. Regulation of Neurogenesis Unit, GIGA-Neurosciences/GIGA-Stem Cells, ULiège, Liège, Belgium; [3]Novel Mediators in Lung Oncogenesis, Inst. Européen de Chimie et Biologie (IECB), Univ. de Bordeaux, Bordeaux, France; [4]Exptl. Oncology, Mol. Oncology Programme, Ctr. Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain

Abstract: ***Aim*** Cdk7 is a serine/threonine kinase which is part of the Cdk-activating kinase (CAK) complex together with cyclin H and Mat1. This protein complex is mostly known for its positive role on proliferation through cell cycle Cdks phosphorylation in both normal and pathological (cancer) conditions. In the developing neocortex, it has been recently demonstrated that Cdk7 contributes to cell cycle progression of neural progenitors. Besides its role on Cdks, CAK complex also regulates transcription both directly by phosphorylating RNA polymerase II, promoting thereby transcription initiation, and indirectly by phosphorylating several nuclear receptors (i.e. RAR-α, RAR-γ, ER-α, PPAR-α and PPAR-γ), leading to specific genes transcription. Despite this plural transcriptional role, CAK complex disruption does not impair global transcription but affects different subsets of genes in specific tissues. Such genes have been identified in numerous proliferative cell types under both normal and pathological conditions. But, up to now, very few studies have investigated CAK complex transcriptional impact in postmitotic cell types, including in the brain. Using a genetic approach, we decided to unravel the role of Cdk7 in postmitotic cortical neurons.
Methods To decipher Cdk7 transcriptional function(s) independently of its role on proliferation, we use a conditional knock-out (Nex^{Cre}) C57BL/6-Sv/129 mice model in which Cdk7, the CAK complex catalytic member, is invalidated in postmitotic cortical and hippocampal neurons. We analyze any cortical phenotype at different time points and check mice behavior. We also perform *in vitro* loss-of-function assays using primary cortical neurons cultures.
Results Our results indicate that Cdk7 conditional invalidation in postmitotic cortical neurons induces cortical layer-unspecific neuronal packing without affecting total number of neurons *in vivo*. This phenotype is associated to dendritic size and complexity impairments in those neurons, observed in both *in vivo* and *in vitro* experiments.
Conclusion Together, our data highlight a role for Cdk7 and the CAK complex in the morphological development of cortical neurons. Further investigations are conducted to determine 1) if the phenotype is restricted to dendrites, 2) the mechanisms underlying this phenotype and 3) the impact of this phenotype on mice behavior.

Disclosures: **S. Verteneuil:** None. **Q. Marlier:** None. **D. Santamaría:** None. **M. Barbacid:** None. **L. Nguyen:** None. **R. Vandenbosch:** None. **B. Malgrange:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.17/A33

Topic: A.05. Axon and Dendrite Development

Support: TWU Department of Biology
Texas Women's University Research Enhancement Program

Title: Localization of prenylated and palmitoylated Cdc42 in neuroblastoma cells

Authors: *K. P. B. KNOWLES, N. G. R. R, D. HYNDS;
Texas Woman's Univ., Denton, TX

Abstract: Cdc42, a small GTPase belonging to the Rho sub-family, acts as a molecular switch for signaling cascades that affect cell motility, morphology, and neural plasticity. Like other GTPases, Cdc42 is active when bound to GTP, facilitated by guanine exchange factors (GEFs), and inactive when bound to GDP, catalyzed by GTPase activating proteins (GAPs). Active GTP-bound Cdc42 can interact with downstream effectors, but another form of regulation occurs by the binding of guanine dissociation inhibitors (GDIs) to inactive GDP-bound Cdc42, sequestering it to the cytosol. The dogma of Rho GTPase activation has been that it must undergo post-translational modification of prenylation to colocalize at the membrane with GEFs. As well as canonical Cdc42, a splice variant found in the brain, bCdc42, differs in the c-terminal exon. Canonical Cdc42 carboxy-terminal ends with -CVLL and bCdc42 ends in -CCIF, the double cysteine at the c-terminal allows the splice variant bCdc42 to undergo both post-translational modifications of prenylation and palmitoylation. There has been speculation on the different signaling functions of the two splice variants and how they localize at the cell membrane.
To investigate the difference in localization between the two splice variants, we first investigated the endogenous proteins present in neuroblastoma cells. We used specific antibodies to detect the canonical form of Cdc42, which is prenylated, and the brain-specific Cdc42 that is prenylated and palmitoylated. Thus far, we found that the canonical Cdc42 transitions between the endoplasmic reticulum and the cell membrane, being evenly dispersed. However at higher concentrations, it localizes near the axon. Brain-specific Cdc42 transitions between the Golgi apparatus to near the dendritic arbors. We hypothesize that bCdc42 palmitoylation allows it to transiently associate with the Golgi apparatus to facilitate dendritic morphogenesis. Defining the roles of the two Cdc42 splice variants will help us better understand why the brain produces two versions of Cdc42 and the implications this has for neurological disorders.

Disclosures: K.P.B. Knowles: None. **N.G.R. R:** None. **D. Hynds:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.18/A34

Topic: A.05. Axon and Dendrite Development

Title: Identification of distinct effects of the G protein-coupled estrogen receptor in embryonic rat hippocampal and cortical cells

Authors: *K. PEMBERTON, F. XU;
Dept. of Biol., St. Louis Univ., Saint Louis, MO

Abstract: Most studies investigating estrogen's effect on the nervous system have focused on the traditional estrogen receptors Estrogen Receptor-α (ERα) and Estrogen Receptor-β (ERβ). The recent identification of the G-Protein Coupled Estrogen Receptor (GPER) suggests knowledge of estrogen's effects may be incomplete. Evidence has suggested GPER may play multiple roles in the nervous system including neuroprotection, neuronal recovery from stroke and degeneration, neuron proliferation, as well as others. A caveat is that most studies have looked at the effect in mature neurons, while GPER's effect during early neuronal development is largely unknown. Here we sought to study the role of GPER activity in neurite outgrowth and synapse formation, as well as their underlying signaling during early development in two distinct brain regions. Embryonic day 18 rat cortical and hippocampal neurons were cultured in estrogen- and phenol red-free medium in the presence of a nonselective estrogen receptor agonist (E2), a selective GPER agonist (G-1), and a selective GPER antagonist (G-15). Neurite outgrowth was measured at 20, 48, 72, and 96 hours in culture using the ImageJ plugin NeuronJ. Synapses were visualized at 7, 14, and 21 days in culture (DIC) by using immunofluorescence of a pre-synaptic marker and dendritic markers or post-synaptic markers. Synapse formation was measured by identifying areas of fluorescent overlap in ImageJ. Calcium imaging was done at 4, 7, 14, and 21 DIC using a fluorescent calcium indicator and signal intensity changes measured in ImageJ. Multi-Electrode array data was measured at 8, 16, 21 DIC and spiking activity was identified using Multi Channel Systems Multi Channel Analyzer software. All data was analyzed using R in RStudio. Our data revealed activation of GPER promoted neurite growth in hippocampal neurons, but not in cortical neurons, although blocking the receptor inhibited neurite growth in cortical neurons. Synapse formation was also altered in a distinct manner between the two brain regions. Furthermore, both calcium imaging data and multi-electrode array data indicate GPER affects neuron signaling differently in these two brain regions. This provides the first evidence that GPER activation may signal differently in distinct brain regions, as well as having different roles in specific regions. Future experiments will further investigate the molecular and cellular mechanisms underlying the pharmacological differences in GPER effects between developing cortical and hippocampal neurons.

Disclosures: K. Pemberton: None. **F. Xu:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.19/A35

Topic: A.05. Axon and Dendrite Development

Title: The roles of Calneuron I in the neurite outgrowth and synaptic transmission in primary cultured rat embryonic cortical neurons

Authors: *H. WU, C.-Y. PAN;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Calneuron I (CalnI), a calmodulin-like protein, binds Ca^{2+} by its two functional EF-hand motifs at the N-terminal and contains a transmembrane segment at the C-terminal. CalnI is mainly expressed in the mouse brain at a late developing stage after P15. Our previous results have shown that CalnI inhibits the voltage-gated Ca^{2+} currents in cultured bovine chromaffin cells. Other reports suggest that CalnI participates in the regulation of Golgi-plasma membrane trafficking and has the best Ca^{2+} affinities, K_d ~ 180 nM, globally comparing with other Ca^{2+} sensors expressed in neurons. However, the roles of CalnI in regulating the neuron differentiation and synaptic transmission are not well-characterized yet. In this study, we overexpressed CalnI and mutants in primary cultured rat embryonic cortical neurons using Amaxa Neucleofector. We first examined the morphology of the transfected neurons using Sholl analysis. The results showed that neurons expressing CalnI has no significant difference from control group expressing GFP in the neurite outgrowth pattern. We will further characterize the effects of CalnI in modulating the synaptic transmission by loading the cultured neurons with Fura-2, a Ca^{2+}-sensitive fluorescence dye, and examined the changes in the intracellular Ca^{2+} concentrations under different stimulations. We will examine the Ca^{2+} responses in the neurons expressing CalnI and neighbor neurons without expressing CalnI to characterize the efficacy in signal transduction from the CalnI-expressing neurons to other post-synaptic neurons. Overall, our study will provide detail information about the roles CalnI in modulating the neurotransmission.

Disclosures: H. Wu: None. **C. Pan:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.20/A36

Topic: A.05. Axon and Dendrite Development

Title: Cortactin deacetylation by HDAC6 and SIRT2 regulates dendritic Golgi polarization and neuronal cell migration during brain development

Authors: *J.-Y. KIM, H. HWANG, H. JEON, J.-Y. LEE, M. KIM, J.-Y. KIM;
Chungnam Natl. Univ., Daejeon, Korea, Republic of

Abstract: Neuronal cell development is important for the proper cell migration and neural circuit formation in brain. Neurons undergo multipolar-to-bipolar transition during radial neuronal migration and develop apical and basal dendrites in the cerebral cortex. Histone deacetylase 6 (HDAC6), of which substrates include acetylated α-tubulin and cortactin, regulates cytoskeletal dynamics in cytoplasm. However, roles of HDAC6 in neuronal cell morphogenesis and migration remain largely unknown. In this study, we investigated whether HDAC6 is required for neuronal cell development during brain development. Knockdown of HDAC6 resulted in abnormal Golgi polarization and defective dendritic growth *in vitro*. Interestingly, overexpression of another cytoplasmic deacetylase SIRT2 rescued the defects of HDAC6 knockdown, suggesting that HDAC6 and SIRT2 may be functionally redundant. Expression of wild type and deacetylation mimetic form of cortactin, but not α-tubulin, suppressed the defects in Golgi polarization and dendritic growth in HDAC6 knockdown neurons, indicating that HDAC6 promotes Golgi polarization and dendritic growth through cortactin deacetylation. *In utero* electroporation with HDAC6 or SIRT2 shRNA resulted in no apparent defect in radial neuronal migration, but double knockdown of HDAC6 and SIRT2 delayed radial neuronal migration in neocortex. In addition, overexpression of acetylation mimetic cortactin 9KQ showed defects in dendritic Golgi polarization and radial neuronal migration. Taken together, this study suggests that cortactin deacetylation by HDAC6 and SIRT2 is essential for neuronal cell development during brain development.

Disclosures: J. Kim: None. **H. Hwang:** None. **H. Jeon:** None. **J. Lee:** None. **M. Kim:** None. **J. Kim:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.21/A37

Topic: A.05. Axon and Dendrite Development

Title: Retinal determinant of prefrontal cortex function and integrity

Authors: *J. ZHAN;
Natl. Inst. of Hlth. and Johns Hopkins Univ., Washington D.C., MD

Abstract: In modern mammals many non-image-forming visual functions, such as circadian photo-entrainment, are the preserve of a subpopulation of retinal ganglion cells (RGCs) that express the photopigment melanopsin (Opn4) and are therefore intrinsically photosensitive (ipRGCs). A diverse bunch, ipRGCs comprise at least five classes distinguished by size, dendritic stratification, electrophysiological properties, and central projections. Recently shown, a circuit linking ipRGCs to the ventromedial prefrontal cortex (vmPFC) via a relay in the dorsal thalamus mediates light's affect on mood. Genetic ablation of these ipRGCs renders animals

invulnerable to mood alterations induced by an aberrant light paradigm. The thalamic relay in this pathway, the perihabenular nucleus (PHb), is the source of dense and previously unknown innervation of the vmPFC. The PHb's innervation field is precisely layers I and III-IV of the infralimbic cortex, home to a resident population of pyramidal neurons distinguished by their exceptional dendritic-arbor plasticity. In these neurons, a wide range of stressors, from forced swimming to physical restraint, are reported to induce dentritic retraction. Given this dynamic phenomenology, we decided to investigate layer III-IV pyramidal neurons in the vmPFC of ipRGC-ablated mice. We found that in the absence of ipRGCs, pyramidal neurons of the vmPFC suffer apical dendritic deficits of a surprising magnitude. Interestingly, mice lacking ipRGCs also exhibited abnormal behaviors during social interactions. Single cell sequencing of vmPFC tissue revealed that many genes enriching biological pathways related to synaptic plasticity and dendritic remodeling were dysregulated. Given these results, we provide evidence for a previously unrecognized role of ipRGCs in providing essential input for maintaining vmPFC function and integrity.

Disclosures: **J. Zhan:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.22/A38

Topic: A.05. Axon and Dendrite Development

Support: NSERC
NSERC-PGS
VSRP

Title: Dendrite self-avoidance is mediated by transient neurite bridges

Authors: ***S. ING-ESTEVES**[1,2], R. FARHOUDI[3], K. P. KORDING[3,4], J. L. LEFEBVRE[1,2]; [1]Neurosci. and Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada; [2]Mol. Genet., Univ. of Toronto, Toronto, ON, Canada; [3]Bioengineering, [4]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Dendritic arbor patterns develop through an iterative process of neurite addition, retraction, and recognition of environmental cues. For some neuron types an important aspect of this process is self-avoidance, in which dendrites belonging to the same neuron avoid each other to prevent overlap and to evenly fill the space. We have shown previously that dendrite self-avoidance is regulated by the clustered Protocadherin proteins (cPcdhs). The cPcdhs are transmembrane molecules proposed to mediate homophilic interactions at the cell membrane to facilitate recognition between contacting neurites. In the absence of cPcdhs the dendrites of

Starburst amacrine cells, a retinal interneuron, fail to self-avoid and form bundles and crossings. However, the cellular events and cues that produce self-avoidant arbors are unknown. To investigate the developmental mechanism of self-avoidance, we used time-lapsed imaging to capture 3D volumes of developing Starburst dendrites in both wildtype and cPcdh deficient mouse retinas. Here, we demonstrate that dendrite self-avoidance occurs in a contact-dependent manner through ‘dendritic bridges’: orthogonal, filopodia-like projections that transiently connect adjacent primary branches. Dendrite bridges continuously form, retract, and re-form within minutes. Quantifying bridge dynamics requires the detection of 'loops' within a dendrite structure making the resulting reconstruction a cyclic graph. To track bridge dynamics, we developed a pipeline to reconstruct dendrites for each timepoint and align the resulting graphs through time. In wildtype neurons we observe rapid bridge dynamics that maintain separation between growing branches. Conversely, in cPcdh deficient neurons dendritic bridge dynamics are altered, contributing to the collapse of developing branches. Together, our results suggest that developing neurons survey the proximity of neighbouring self-dendrites through Pcdh-dependent interactions from contacting bridges. We propose that these dendritic bridge-to-branch contacts elicit the cell-intrinsic self-avoidance signals that guide branch growth and spacing.

Disclosures: S. Ing-Esteves: None. **R. Farhoudi:** None. **K.P. Kording:** None. **J.L. Lefebvre:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.23/A39

Topic: A.05. Axon and Dendrite Development

Support: T32 G007288-44

Title: Characterizing novel pathways of dendritic tiling in *C. elegans*

Authors: *M. TRIVEDI, L. A. HERNANDEZ, H. E. BUELOW;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Neurons rely on dendrites for the acquisition of sensory and synaptic input from their particular receptive fields. Findings of aberrant dendritic morphology in disorders such as autism spectrum disorder (ASD) and schizophrenia highlight the importance of understanding how complex dendritic arbors are developed and maintained. During development, one of the goals of dendritic outgrowth is non-redundant coverage of a receptive field, which requires the avoidance of other dendrites both from the same neuron (self-avoidance) and from others (tiling). While tiling is a conserved property of many nervous systems, the molecular mechanisms by which it is established remain unclear. The goal of this project is to characterize the mechanisms of

dendritic tiling using the multi-dendritic FLP and PVD mechanosensory neurons of *C. elegans* as a model. The dendritic arbor of FLP covers the head of the worm while the arbor of PVD covers the body. The mechanism by which these neurons establish distinct non-overlapping receptive fields remains unknown. Using an unbiased forward genetic approach, we isolated a mutant allele in *unc-33*, which displays altered FLP and PVD receptive field sizes. *Unc-33* encodes a member of the Collapsin Response Mediator Protein (CRMP) family, which regulate axon outgrowth and morphology by binding and organizing tubulin heterodimers. I hypothesize that *unc-33/CRMP* acts to define the border between FLP and PVD by organizing microtubules in outgrowing dendrites. Furthermore, I hypothesize that unbiased forward genetic approaches will uncover additional regulators of FLP and PVD tiling.

Disclosures: **M. Trivedi:** None. **L.A. Hernandez:** None. **H.E. Buelow:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.24/A40

Topic: A.05. Axon and Dendrite Development

Title: Cap1 structure regulates dendritic complexity through post transcriptional gene regulation

Authors: *Y.-S. HUANG[1], L.-L. LEE[1], P.-L. CHENG[2];
[1]Academia Sinica/Institute of Biomed. Sci., Taipei, Taiwan; [2]Academia Sinica/ Inst. of Mol. Biol., Taipei, Taiwan

Abstract: The 5′-end of eukaryotic mRNAs is capped with a 7-methylguanosine (m^7G) during the initial phase of transcription. Unlike yeast mRNAs which contain only primitive m^7G cap (i.e. cap0, $m^7GpppNN$, N: any nucleotide), the cap structure in higher eukaryotic organisms is more complicated with additional 2′-O-ribose methylation at the first and second nucleotides by cap methyltransferase (CMTR) 1 and 2, respectively. Cap0 is essential for nuclear export, stability and cap-dependent translation of mRNAs but the function of 2′-O-ribose methylation in mRNAs (cap1, m^7GpppN_mN; cap 2, m^7GpppN_mNm) is less clear. A previous study reported that knockdown (KD) of CMTR1 in human cell lines evokes innate immune responses because cap1-deficient mRNAs are recognized as non-self molecules by the RNA sensor, retinoic acid-inducible gene-I (RIG-I). Despite this housekeeping function, we hypothesize that cap1 modification may promote stability and/or translational efficiency of specific mRNAs, so we investigate CMTR1 function in neurons where rich post-transcriptional regulations control molecular diversities to support their complex morphologies and functions. Using KD approach in cultured neurons, we found that CMTR1 deficiency impairs the development of dendritic complexity, which is caused by altered posttranscriptional gene expression rather than innate immune response. For example, the expression of calcium/calmodulin-dependent protein kinase

II α subunit (CaMKIIα), which plays an important role in neuronal maturation, is downregulated in CMTR1-KD neurons. Whether and how cap1 modification affects target-specific gene expression is currently under investigation.

Disclosures: Y. Huang: None. **L. Lee:** None. **P. Cheng:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.25/A41

Topic: A.05. Axon and Dendrite Development

Support: KAKENHI 17K16409

Title: Slit2 is preferentially expressed in the higher-order association area of primate cortex

Authors: *T. SASAKI[1], Y. KOMATSU[2], T. YAMAMORI[3];
[1]Univ. of Tsukuba, Tsukuba, Japan; [2]Natl. Inst. Basic Biol, Okazaki, Japan; [3]BSI, RIKEN, Wako, Japan

Abstract: To elucidate the molecular basis for the specialization of cortical architectures, we searched for genes differentially expressed among neocortical areas of Old World monkeys. We previously reported that *SLIT1*, an axon guidance molecule, is abundant in the prefrontal cortex but with developmentally related changes. SLIT is a chemorepellent guidance molecule, which is well conserved in various species. The chemorepellent effect of SLIT is mediated by receptor, Roundabout (ROBO). *In situ* hybridization analysis revealed that *SLIT1* mRNA was mainly distributed in the middle layers of most cortical areas, robustly in the prefrontal cortex and faintly in primary sensory areas (Sasaki et al., 2010). Our comprehensive expression analyses of other *SLIT* (*SLIT2* and *SLIT3*) mRNAs showed enriched expression in the higher-order association areas with a distinct laminar pattern. Among them, the *SLIT2* mRNA expression was high in layers II, III and V in the prefrontal association area. On the other hand, the excitatory population that expressed weak *SLIT2* mRNA signals was restricted to the upper part of the supragranular layers of the primary visual area. These patterns are reminiscent of those of *RBP4* and *PNMA5* mRNAs, which were identified as an association area-enriched gene (Komatsu et al., 2005; Takaji et al., 2009; Yamamori 2011). Scattered signals of *SLIT2* mRNA were identified in layers III-VI were in *GAD67*-mRNA-positive inhibitory neurons. Double ISH analysis showed that *SLIT2* and *RBP4* mRNAs were colocalized in the cortical neurons. Thus, *SLIT2, RBP4* and *PNMA5*, whose expressions show the *RBP4*-like expression in the cortex, may all exert influence over a similar type of cortical neuron.

Disclosures: T. Sasaki: None. **Y. Komatsu:** None. **T. Yamamori:** None.

Poster

029. Molecular Mechanisms of Axon and Dendrite Development

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 029.26/A42

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01NS109176
NIH Grant R21NS101450

Title: Characterizing the role of Arl4c during dendrite morphogenesis in hippocampal pyramidal neurons and its regulation by CRL5

Authors: *J. S. HAN, S. SIMO;
Cell Biol. and Human Anat., Univ. of California Davis, Davis, CA

Abstract: During development, neurons produced by hippocampal neuronal progenitors migrate to their proper destination where they stop and integrate into the developing neuronal network. Although these are essential steps for proper hippocampal development and function, a complete portrait of the signaling pathways involved and how they are regulated remains incomplete. The Cullin-5 RING E3 ubiquitin ligase (CRL5) complex regulates various important signaling pathways for neuron positioning, cell polarity and dendritogenesis in the developing cerebral cortex, cerebellum and retina. However, the role of CRL5 in the hippocampus has not been characterized yet. Thus, understanding the signaling pathways regulated by CRL5 in the hippocampus will provide much needed information on how CRL5 participates in hippocampal development and likely in other areas of the central nervous system. Here, we show that CRL5 regulates the expression of ADP-ribosylation factor-like 4c (Arl4c) in the telencephalon. Arl4c is a member of the Arf family of GTP-binding proteins and its biological role in the brain is unknown. We found that Arl4c expression increases during early postnatal stages in the hippocampus, peaks around postnatal day 8 (P8) and decreases at juvenile stages. Interestingly, this change in protein levels is not a consequence of differential *arl4c* expression. Our histological data shows predominant expression of Arl4c in the Cornu Ammonis (CAs), particularly in the dendritic tree of pyramidal neurons. Importantly, we show that Arl4c is localized at the plasma membrane and in cytoplasmic vesicles of pyramidal neurons. These data suggest that Arl4c expression is post-transcriptionally controlled by CRL5 and its activity is regulated by changes in its subcellular localization. We demonstrate that depletion of Arl4c promotes neurite complexity while overexpression shows the opposite effect in hippocampal pyramidal neurons using Sholl analysis. Furthermore, our data indicate that Arl4c affects actin remodeling via small GTPase regulation. Finally, we show that in the absence of CRL5 activity, the expression of other Arl4c-dependent signaling factors, including Cytohesin-1/3, Arf6, and Frmd4A, was also deregulated. Together, these data indicate that CRL5 controls dendrite

arborization during hippocampal development through regulation of Arl4c and its associated signaling effectors and suggest that CRL5 is a novel regulator of hippocampal morphogenesis.

Disclosures: **J.S. Han:** None. **S. Simo:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.01/A43

Topic: A.07. Developmental Disorders

Support: CIHR 374967

Title: Unstable stalled polysomes underlie dysregulated protein synthesis in human IPSC-derived fragile X neurons

Authors: *J. J. LANGILLE, G. MAUSSION, C. ROCHA, T. DURCAN, W. SOSSIN; McGill Univ., Montreal, QC, Canada

Abstract: The brain captures information through a remodeling of synapses. Regulated protein synthesis makes this remodeling long-term, and stalled polysomes are one mechanism for achieving this regulation. Stalled polysomes are large granules located predominately in neuronal processes comprised of ribosomes, assembled around messenger RNA and translationally paused by RNA binding proteins. How these protein synthetic structures operate in human neurons and in disease has been largely a mystery. Using stem cells, our research demonstrates stalled polysomes in healthy human neurons. Comparatively, neurons derived from the stem cells of patients with Fragile X syndrome, a neurodevelopmental disorder defined by a silencing of the gene encoding Fragile X mental retardation protein (FMRP), have fewer stalled polysomes. Moreover, neurons from these patients have elevated levels of the special form of translation stalled polysomes mediate and of Map-1b protein, the mRNA of which is thought to in stalled polysomes. These observations suggest that absent repression by FMRP, stalled polysomes release prematurely and translation of synapse weakening mRNAs increases. Indeed, re-instatement of FMRP in Fragile X neurons reduces neurite levels of stalled polysome mediated translation. Thus, neurons acquired from human patient stem cells have stalled polysomes which are dysregulated in brain disease.

Disclosures: **J.J. Langille:** None. **G. Maussion:** None. **C. Rocha:** None. **T. Durcan:** None. **W. Sossin:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.02/A44

Topic: A.07. Developmental Disorders

Support: 81527901
16JC1420501
31625013
91732302
XDBS01060200
2018SHZDZX05
2017LH036

Title: The expression pattern and neurobiological functions of chinese autism spectrum disorder risk genes

Authors: *K. YANG, J. WANG, Y. ZHANG, Z. QIU;
Inst. of Neuroscience, Chinese Acad. of Scie, Shanghai, China

Abstract: Autism spectrum disorder (ASD) is a series of symptoms classified as neurodevelopmental disorders with unclear pathological mechanisms. Individuals diagnosed with autism spectrum disorder perform with two types of symptoms: problems in social communication, and restricted, repetitive patterns of behavior. To date, hundreds of ASD risk genes have been reported. However, the expression pattern and neurobiological functions of ASD risk genes in developing human brain is still elusive. In this project, we first discovered 122 *de novo* mutations from 200 Chinese ASD simplex trios with applying whole-exome-sequencing combined with Sanger-sequencing-validation. The cohort with genes carrying *de novo* mutations is hereafter termed as the Chinese ASD risk gene list (CARL). Then, in compare with SFARI genes cohort, we analyzed the consecutive expression pattern of both CARL genes and SFARI genes in developing human brain. Interestingly, we found genes from both of these cohorts were highly expressed before ASD core symptoms emerging, while globally decreased yet fluctuated during the period of ASD. This observation indicated that CARL genes and SFARI genes were generally required in early neurodevelopmental events and needed to be precisely regulated during rapid developing stages of neural system. Next, we explored biological functions of several CARL genes or SFARI genes with cultured mouse cortical neuron system *in vitro* and mouse embryonic electroporation system *in uterus*. Surprisingly, we discovered different specific neurobiological phenotypes that strongly correlated to severity of ASD or severity of intellectual developmental delay, independently. What was more, these pathological phenotypes mimicked by mutants of CARL genes or SFARI genes matched with symptoms of ASD individuals to a

great extent. In all, this work described a general expression pattern of ASD risk genes, showing emerging evidences that explain for contribution of *de novo* mutations to ASD symptoms. Our findings provided new knowledge to better understand inner mechanism of ASD.

Disclosures: K. Yang: None. **J. Wang:** None. **Y. Zhang:** None. **Z. Qiu:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.03/A45

Topic: A.07. Developmental Disorders

Support: MH116453-01A1
MH112237
MH108842
DA037618

Title: Chemogenetic activation of prefrontal cortex in Shank3-deficient mice ameliorates social deficits, NMDAR hypofunction and SGK2 downregulation

Authors: *L. QIN, K. MA, Z. YAN;
Physiol. and Biophysics, State Univ. of New York (SUNY) at Buffalo, Buffalo, NY

Abstract: A hallmark for autism spectrum disorder (ASD) is social deficits and restricted & repetitive behaviors. Presently, there is no known cure for ASD. Haploinsufficiency of the *SHANK3* gene is causally linked to ASD in human genetic studies. *Shank3*-deficient mice exhibit autism-like deficits, significantly diminished N-methyl-D-aspartic acid (NMDA) receptors synaptic function in prefrontal cortex (PFC). To identify a novel approach for the treatment of autism-like social deficits, we decide to use the chemogenetic tool, designer receptors exclusively activated by designer drugs (DREADDs), which enables the remote, noninvasive and long-lasting modulation of cellular activity and signal transduction in discrete neuronal populations in vivo. We found selectively activating PFC pyramidal neurons with a CaMKII-driven hM3D (Gq) DREADD could rescue autism-like social behavioral deficits, elevate diminished NMDARs and facilitate AMPARs synaptic function and normalize the decreased serum- and glucocorticoid-inducible kinase 2 (SGK2) expression. Competitive blocking the interaction of all SGK isoforms with their endogenous substrates substrate peptide (RPRAATF) abolished the rescued autism-like social deficits and elevated the NMDARs function in *Shank3*-deficient mice by chemogenetic activation PFC pyramidal neurons and induced autism-like social deficits and decreased the NMDARs and AMPARs function in WT mice. Inactivation of PFC pyramidal neurons in WT mice with hM4D (Gi) DREADD is insufficient to induce changes in social behaviors and SGKs expression. These results suggest that chemogenetic activation

PFC pyramidal neurons increases the trafficking and function of NMDARs through SGK2, thereby rescuing autism-like social deficits, which helps to identify novel molecular and cellular targets for the design of novel therapeutic strategies for ASD patients carrying *Shank3* mutations.

Disclosures: L. Qin: None. **K. Ma:** None. **Z. Yan:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.04/A46

Topic: A.07. Developmental Disorders

Support: Jerome Lejeune Foundation

Title: Autism-associated δ-catenin G34S mutation promotes GSK3β-mediated premature δ-catenin degradation inducing neuronal dysfunction

Authors: *S. KIM[1], K. A. NIP[1], M. SATHLER[1], J. SHOU[2];
[2]Dept. of Biomed. Sci., [1]Colorado State Univ., Fort Collins, CO

Abstract: δ-catenin is a crucial component of a synaptic scaffolding complex, which regulates synaptic structure and function in neurons. Loss of δ-catenin function is strongly associated with severe autism spectrum disorder (ASD) in female-enriched multiple families. In particular, a G34S (Glycine 34 to Serine) mutation in the δ-cateningene has been identified in ASD patients and suggested to exhibit loss-of-function. The G34S mutation is located in the amino terminal region of δ-catenin, where there are no known protein interaction domains and post-translational modifications. Notably, the Group-based Prediction System predicts that the G34S mutation is an additional target for GSK3β-mediated phosphorylation, which may result in protein degradation. Therefore, we hypothesize the G34S mutation accelerates δ-catenin degradation, resulting in loss of δ-catenin function in ASD. Indeed, we found significantly lower G34S δ-catenin levels compared to wild-type (WT) δ-catenin when expressed in cells lacking endogenous δ-catenin, which is rescued by genetic inhibition of GSK3β. By using Ca2+imaging in cultured mouse hippocampal neurons, we further revealed overexpression of WT δ-catenin is able to significantly increase neuronal Ca2+activity. Conversely, Ca2+activity remains unaffected in G34S δ-catenin overexpression, which is reversed by pharmacological inhibition of GSK3β using lithium. This suggests the G34S mutation of δ-catenin provides an additional GSK3β-mediated phosphorylation site, which could promote δ-catenin premature degradation, resulting in loss-of-function effects on neuronal Ca2+activity in ASD. In addition, inhibition of GSK3β activity is able to reverse G34S-induced loss of δ-catenin function. Thus, inhibition of GSK3β may be a potential therapeutic treatment for δ-catenin-associated ASD patients.

Disclosures: **S. Kim:** None. **K.A. Nip:** None. **M. Sathler:** None. **J. Shou:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.05/A47

Topic: A.07. Developmental Disorders

Support: HIAS#18001 (to GJ Blatt)
Hussman Foundation for Autism Research

Title: Depressed dopamine D1 receptor expression in Broca's area in autism

Authors: *J.-J. SOGHOMONIAN[1], K. ZHANG[1], M. KATCHADOORIAN[2], N. KHANNA[2], C. BRANDENBURG[3], G. J. BLATT[3];
[1]Boston Univ. Sch. of Med., Boston, MA; [2]Boston Univ., Boston, MA; [3]Hussman Inst. For Autism, Baltimore, MD

Abstract: Autism spectrum disorders (ASD) are paralleled by neurochemical imbalances including neurotransmitter imbalances in several brain regions. In particular, an earlier study has found abnormalities in the expression of serotonin and dopamine transporters in the autism brain*(*Arch Gen Psychiatry. 2010;67(1):59-68). Broca's area in the left prefrontal cortex is a major language motor region and it could be involved in language skill differences in ASD. Dopamine is an important neuromodulator that has been shown to drive left-lateralization of functional activity in cortical language area (J Comp Neurol. 2018; 526:920-931). The objective in our study was to determine the possibility that dopaminergic imbalances occur in Broca's area in ASD that could explain some of the language differences. Post-mortem human brains from controls (n=21) and autism (n=22) subjects were sectioned at the level of Broca's area and processed for radioisotopic in situ hybridization histochemistry with a cRNA probe selective for the human dopamine D1 (Drd1) receptor and then processed for radioautography. First, the mRNA labeling was analyzed at the regional level by densitometry on x-ray films and secondly at the cellular level on emulsion radioautographs. Results show that the mRNA levels of Drd1 were significantly lower in Broca's area of ASD compared to control brains. This effect was documented at the regional level by including the whole cortical thickness and was also documented at the single cell level on emulsion radioautographs. These findings suggest that changes in the dopaminergic system in Broca's area may be involved in language and communication deficits in ASD. It is unclear if these effects are related to the decreased grey matter volume documented in several regions of the prefrontal cortex (Front. Hum. Neurosci., 04 August 2017. https://doi.org/10.3389/fnhum.2017.00395).

Disclosures: **J. Soghomonian:** None. **K. Zhang:** None. **M. Katchadoorian:** None. **N. Khanna:** None. **C. Brandenburg:** None. **G.J. Blatt:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.06/A48

Topic: A.07. Developmental Disorders

Support: Brown University New Frontiers Award
Brown University Seed Award

Title: Role of matrix metalloproteinase-9 (MMP9) in pathophysiology of neurodevelopmental disorders in *Xenopus laevis* tadpoles

Authors: ***S. GORE**[1], A. DELGADO CARRION[1], L.-C. HUANG[2], E. JAMES[1], A. BERGHELLA[1], H. CLINE[2], C. AIZENMAN[1];
[1]Neurosci., Brown Univ., Providence, RI; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social and cognitive functions. Prenatal exposure to valproic acid (VPA), a common antiepileptic drug, results in ASD in humans and in neurodevelopmental abnormalities in other animal models. Similarly, exposure to VPA during a critical developmental period in Xenopus tadpoles causes behavioral and electrophysiological abnormalities consistent with hyperconnected neural networks. VPA exposure results in upregulation of MMP9 levels in tadpole brains, suggesting a role of MMP9 in VPA-induced neurodevelopmental disorders. MMP9 is a matrix metalloproteinase that cleaves various components of extracellular matrix, enabling synaptic and circuit level reorganization. We investigated the role of MMP9 in the early formation of neural circuitry within the Xenopus optic tectum. We predicted that upregulation of MMP9 mimics VPA-induced effects while downregulation would result in the rescue of these effects. The upregulation of MMP9 was achieved by whole tissue electroporation of an MMP9 overexpression construct while downregulation was achieved using either a pharmacological agent or by using an antisense morpholino (MO) against MMP9. Overexpression of MMP9 resulted in significant increase in the frequency of sEPSCs and sIPSCs, similar to the VPA-effects while inhibition of MMP9 rescued these effects, without altering the basal transmission. MMP-9 levels are elevated after patterned visual exposure, resulting in elevated tectal cell dendritic growth. This growth could be arrested by using MMP9 inhibitors, indicating that transient expression of MMP9 promotes growth. MMP9 is known to activate BDNF, a neurotrophin essential for neuronal development, and dysregulation of BDNF is implicated in several neurodevelopmental disorders. We show that pharmacological inhibition of BDNF rescues VPA-induced effects. Taken together, our findings suggest that early VPA exposure

results in chronically elevated BDNF levels mediated via MMP9. This results in hyperconnected neural networks which elicit ASD-related behaviors. This study demonstrates that disregulation of MMPs during early brain development can be an important contributor to the etiology of neurodevelopmental disorders.

Disclosures: S. Gore: None. **A. Delgado Carrion:** None. **L. Huang:** None. **E. James:** None. **A. Berghella:** None. **H. Cline:** None. **C. Aizenman:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.07/A49

Topic: A.07. Developmental Disorders

Support: NIH Grant 1 R15 NS101608-01A1

Title: Investigating how the chromatin remodeling CHD protein, Kismet, affects BMP signaling and synaptic function

Authors: *R. A. SMITH[1], F. L. LIEBL[2];
[1]Biol. Sci., [2]Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: CHD7 and CHD8 are chromatin remodeling proteins that regulate gene expression. Alterations in gene expression are needed for synaptic plasticity and long-term memory. Mutations in these chromatin remodeling genes occur in Autism Spectrum Disorders and CHARGE Syndrome. The *Drosophila* homolog of CHD7 and CHD8 is Kismet (Kis). We found that *kis* mutant synapses have increased BMP signaling, increased levels of cell adhesion molecules, and decreased endocytosis. These pathways/proteins are vital for synapses to efficiently communicate and remodel. We sought to better characterize the *kis* mutant phenotypes. There was no significant difference in nervous wreck levels at the synapse of *kis* mutants compared with controls indicating that the increase in BMP signaling found in *kis* mutants isn't likely the result of deficient endocytosis of BMP receptors by nervous wreck. We did find, however, that BMP signaling and the levels of the postsynaptic GluRIIA subunit were increased in neuroligin overexpression mutants. These data suggest that neuroligins may be regulating BMP signaling. To see if pathways that crosstalk with BMP signaling were altered, we examined Frizzled, which is a Wnt receptor, levels and found that they are reduced in *kis* mutants. Future experiments will examine *kis* mutant transcript levels of different BMP pathway components to determine if *kis* is transcriptionally regulating BMP signaling. These data will help us better understand the importance of chromatin remodeling for synaptic structure and function and the molecular changes correlated with neurodevelopmental disorders.

Disclosures: **R.A. Smith:** A. Employment/Salary (full or part-time):; SIUe. 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. **F.L. Liebl:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.08/A50

Topic: A.07. Developmental Disorders

Title: Microbiome derived metabolites associated with autism spectrum disorder negatively impact multiple aspects of neuronal development and functionality

Authors: *K. W. TANG[1], R. GRAF[1], D. DONABEDIAN[1], S. RAO[1], N. CALLIZOT[2], M. COMBES[2], A. HENRIQUES[2], S. CAMPBELL[1];
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Abstract: A strong association between microbiome abnormalities and autism spectrum disorder (ASD) has been demonstrated; however, the pathological mechanisms connecting changes in the gut to those in the brain have yet to be fully elucidated. We hypothesize that bacteria-derived metabolites can disrupt neuronal development and function. To test our hypothesis we focused on 4-ethylphenylsulfate (4-EPS) and indoxyl sulfate (IS), two metabolites known to be elevated in ASD patients. Deficits in myelination, loss of white matter and defects in the function and regulation of synapses have been reported in ASD patients and are believed to be foundational contributors to core ASD behavioral abnormalities. To interrogate these pathologies we leveraged primary cell cultures from rat brain containing either neurons and oligodendrocytes to study neurogenesis and myelination, or hippocampal neurons to study synaptogenesis. Cell cultures were derived from 17-day old rat fetuses. Brain samples were first treated with trypsin to yield a cell suspension. Following further mechanical dissociation neurons and oligodendrocytes, or hippocampal neurons, were isolated and seeded into 96 well-plates pre-coated with poly-L-lysine and laminin, and maintained at 37°C in a humidified incubator during treatment with either 4-EPS of IS. After treatment we adopted an immunostaining approach to identify; oligodendrocyte precursor cells, differentiating oligodendrocytes, mature oligodendrocytes, neurons or synapses. Following appropriate counter-staining images were captured and quantitated using an ImageExpress instrument. Our results demonstrate that IS treatment significantly attenuated ($p < 0.05$); neurite outgrowth, axon formation, proliferation and differentiation of oligodendrocyte precursor cells, oligodendrocyte maturation, axonal myelination and reduced synapse density. Similarly, 4-EPS treatment significantly attenuated neurite sprouting and outgrowth, and axon formation. Interestingly, 4-EPS treatment

significantly increased numbers of mature oligodendrocytes, however, this was not associated with a concomitant increase in axonal myelination. Our data demonstrate that exposure to bacteria-derived metabolites such as 4-EPS and IS negatively impact multiple aspects of neuronal development and functionality. These data highlight a novel therapeutic opportunity that targets the microbiome to develop new medicines that address core behavioral symptoms in ASD patients.

Disclosures: K.W. Tang: None. **R. Graf:** None. **D. Donabedian:** None. **S. Rao:** None. **N. Callizot:** None. **M. Combes:** None. **A. Henriques:** None. **S. Campbell:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.09/A51

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH112714
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The Nancy Lurie Marks Family Foundation
Landreth Foundation
Autism Speaks/National Alliance for Autism Research
Simons Foundation

Title: Increasing UBE3A in substance P (Tac1) or progesterone receptor (Pgr) expressing neurons of VMHvl heightens aggression

Authors: *Y. NONG, D. STOPPEL, M. JOHNSON, M. BOILLOT, J. TODOROVIC, X. ZHOU, M. NADLER, I. NAGAKURA, K. EKKEHARD, M. ANDERSON;
Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Heightened aggression is a common comorbidity of autism spectrum disorder (ASD) including excessive tantrums, irritability, and self-injurious aggressive behaviors that often requires medical treatment. Yet the molecular and neuronal circuit mechanisms underlying the ASD-associated aggression and treatment response remains unknown. We previously reported (SFN 2018) that transgenic *Ube3a* mice (*Ube3a2x* mice, modeling ASD due to maternal *15q11-13* triplication), compared with wild type littermates, display increased total attack time and attack numbers in the resident intruder task. We also found that increasing UBE3A in glutamatergic neurons of ventral lateral subdivision of ventral medial hypothalamus (VMHvl) is sufficient to elevate aggression. In the current study, we further define the neuronal subpopulation in VMHvl where increased *Ube3a* heightens aggression. In VMHvl, 90% of

neurons are glutamatergic, and can be further divided into subpopulations defined by specifically expressing neuronal markers such as the progesterone receptor (*Pgr*), substance P (*Tac1*) or oxytocin receptor (*Oxtr*). To explore the role of *Pgr+*, *Tac1+* or *Oxtr+* neurons in regulating aggressive behavior, we applied *Cre*-targeted chemogenetics and stereotactically injected *AAV-hSyn-DIO-hM3D(Gq)-mCherry* virus into VMHvl of *Pgr-Cre*, *Tac1-Cre* or *Oxtr-Cre* male mice. Four weeks after the virus injection, we performed a standard resident-intruder aggression test to measure the aggression behavior in the virus-injected mice. We found that administering CNO (1 mg/kg i.p.) dramatically increases attack behavior in VMHvl *Tac1+* and *Pgr+* neurons with a smaller effect when targeted to *Oxtr+* neurons. Thus, chemogenetic activation of *Tac1+*, *Pgr+*, or *Oxtr+* VMHvl neurons is sufficient to increase aggression. To determine the neuronal subpopulations in VMHvl where increased Ube3a in these neurons heightens aggression, we stereotactically injected *AAV-hSyn-DIO-Ube3a* virus into VMHvl of *Pgr-Cre*, *Tac1-Cre* or *Oxtr-Cre* male mice. We found that increasing *Ube3a* in *Tac1+* or *Pgr+*, but not in *Oxtr+* neurons increases aggression. Further, when co-injecting inhibitory *AAV-DIO-hM4D(Gi)-mCherry* viruses with *AAV-hSyn-DIO-Ube3a* viruses into VMHvl of *Tac1-Cre* mice, CNO (1 mg/kg i.p.) reversed the heightened aggression due to increased *Ube3a* in *Tac1+* neurons. The results indicate increasing *Ube3a* in the *Tac1+* or *Pgr+* expressing subset of neurons in VMHvl increases aggression, and further confirm the role for VHMvl rather than VMHc/dm in promoting aggression since *Pgr* is expressed selectively in VHMvl.

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Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.10/A52

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 MH107223

Title: Altered histone deacetylase 4 localization in the maternal immune activation mouse model

Authors: *L. BERGDOLT[1], P. COIRO[1], Y. JUNG[1], A. DUNAEVSKY[2];
[2]Dept. of Neurolog. Sci., [1]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Epidemiologic studies have demonstrated an association between prenatal infection and increased risk for neurological disorders such as autism spectrum disorder and schizophrenia in the offspring. Using a mouse model of maternal immune activation (MIA) in which the viral mimic Poly(I:C) is used to induce an immune response in pregnant dams, we and others have

reported structural and functional synaptic deficits in the offspring, yet the molecular mechanisms underlying these deficits remain unknown. We have found increased nuclear accumulation of histone deacetylase 4 (HDAC4), a protein known to regulate expression of synaptic genes and synaptic function, in the cortex of MIA offspring. MIA offspring have decreased cortical expression of calcium/calmodulin-dependent kinase II and cyclin-dependent kinase-like 5, two kinases which phosphorylate HDAC4 which is necessary for its nuclear export. We are working to further characterize alterations in HDAC4 localization throughout the brain of MIA offspring. We have confirmed that HDAC4 is expressed in pyramidal neurons, parvalbumin-positive and somatostatin-positive interneurons, and astrocytes in the cortex. We are determining if altered HDAC4 localization with MIA is cell type specific. In addition, we are examining the extent of HDAC4 mislocalization in the hippocampus and cerebellum - two regions which have been demonstrated to have synaptic impairments in MIA offspring and are implicated in the circuits underlying reported behavioral abnormalities in MIA offspring. We have found an inverse relationship between nuclear HDAC4 and synaptic density of cortical neurons both in vitro and in vivo. Restoration of HDAC4 localization with a high concentration of HDAC4/5 inhibitor in vitro has a significant main effect on synaptic density. Ongoing work will determine if lower doses have a similar effect in vitro. In addition, we will determine if HDAC4 inhibition in vivo ameliorates synaptic impairments and prevents behavioral alterations in MIA offspring.

Disclosures: L. Bergdolt: None. **A. Dunaevsky:** None. **P. Coiro:** None. **Y. Jung:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.11/A53

Topic: A.07. Developmental Disorders

Support: Openminds 620229

Title: A novel autism-associated mutation in UBLCP1 leads to perturbed proteostasis

Authors: *J. SOUEID, Z. HAMZE, D. DAOU, R.-M. BOUSTANY;
American Univ. of Beirut, Beirut, Lebanon

Abstract: Autism spectrum disorder (ASD) is characterized by difficulties in social relatedness, communication, and behavior. Prevalence estimates of ASD in the general population have increased over the last 20 years. Significant shared ancestry and high rates of consanguinity makes the Lebanese population ideally suited for genetic analysis of inherited causes and autosomal recessive ASD susceptibility genes. Whole Exome Sequencing revealed in one family a significant mutation in Ubiquitin-like domain-containing CTD phosphatase 1 (UBLCP1) gene.

UBLCP1 dephosphorylates 26S nuclear proteasomes, preventing assembly of the core and regulatory particles, thereby decreasing their proteolytic activity. This *de novo* missense mutation (g. 158710261CAAAG>C) is predicted to generate a stop codon that interrupts the protein within the phosphatase domain. The disease mutation segregated normally in the family and accounted for <1% rate in the Lebanese population and in Western databases. Compared to normal controls, cultured fibroblasts obtained from the autistic patient bearing this mutation showed a significant increase in proteasome activity which was reflected in decreased ubiquitinated protein levels. A differential RNA expression of 26S Proteasome Subunits (PSMA1, PSMC4, PSMD2) and E3-ligases (UBE3A, SYVN1) was observed compared to normal controls. In parallel, we investigated the anatomical characterization of UBLCP1 in adult mouse brain. UBLCP1 is widely expressed in the brain with a strong signal in the olfactory bulb, the cortex, the diencephalon, the hippocampus, the mesencephalon, the cerebellum, and the rhombencephalon. No expression was observed in the association area of the cortex, the reticular formation, fiber tracts, and the corpus callosum. UBLCP1 localized to the nucleus of MAP2-expressing neurons in the brain, and dopaminergic TH-positive neurons in the ventral tegmental area. Some UBLCP1-positive cells do not express MAP2. We hypothesize that UBLCP1 could be widely expressed in different cell populations of the brain. Our data support current studies suggesting that dysfunctional proteostasis is a common consequence of several genetic mutations linked to ASD. Studies on UBLCP1 mutant induced pluripotent stem cells (iPSCs) are currently undergone to investigate perturbed proteostasis implication in several neuronal processes including neurogenesis, dendritic spine structure, synaptic activity, and the regulation of synaptic strength. This approach will help in the visualization of the larger picture in order to detect common deregulated pathways implicated in the disease, and pave the way for targeted drug development.

Disclosures: **J. Soueid:** None. **Z. Hamze:** None. **D. Daou:** None. **R. Boustany:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.12/A54

Topic: A.07. Developmental Disorders

Support: MRC Grant MR/K022377/1
SFARI 344763
Guy's and St. Thomas's Charity Prize Funding

Title: Altered synaptic development in a heterozygous Chd8 mouse model of autism spectrum disorder

Authors: *R. A. ELLINGFORD[1,2], E. RABESHALA DE MERITENS[1], R. SHAUNAK[1], L. NAYBOUR[2], M. BASSON[2], L. C. ANDREAE[1];
[1]Ctr. for Developmental Neurobio., [2]Ctr. for Craniofacial & Regenerative Biol., King's Col. London, London, United Kingdom

Abstract: De novo loss of function mutations within chromodomain helicase DNA-binding protein 8 (*CHD8*) cause autism spectrum disorder (ASD) with high penetrance. However, the pathological mechanism linking such mutations with the development of ASD is undetermined. We generated a *Chd8* heterozygous mouse line (*Chd8*$^{+/-}$) which shows dysregulated expression of genes vital for synapse and neural circuit formation within the cerebral cortex. Altered levels of excitatory and inhibitory synapse formation (E:I balance) within the cerebral cortex has been proposed as a convergent mechanism through which multiple subtypes of ASD can manifest in humans, providing onus for a detailed examination of synaptic integrity across neurodevelopment in *Chd8*$^{+/-}$ mice. Whole-cell voltage clamp recordings of layer V/VI cortical pyramidal neurons were performed within acute brain slices in order to compare miniature excitatory and inhibitory postsynaptic currents (mEPSCs & mIPSCs) between *Chd8*$^{+/-}$and wildtype mice across early neurodevelopment. *Chd8*$^{+/-}$ mice displayed subtle variations in excitatory synaptic transmission at neonatal stages followed by reduced mEPSC amplitude and frequency at weaning age, indicative of reduced formation of excitatory synapses. Conversely *Chd8*$^{+/-}$ mice showed highly variable levels of inhibitory synaptic transmission at neonatal stages followed by increased mIPSC frequency at weaning age. Interestingly, these changes appeared to be significantly more pronounced in male *Chd8*$^{+/-}$ mice than females, suggesting that reduced expression of *Chd8* has sexually dimorphic effects on synaptic development. Further morphological analysis of the density of excitatory and inhibitory synapses revealed that these changes represent genuine alterations to synapse number. Additionally, layer V/VI cortical projection neurons within *Chd8*$^{+/-}$ mice were found to have unchanged dendritic arborisation, passive membrane properties and action potential firing characteristics. We therefore conclude that reduced expression of *Chd8* specifically impacts synaptic development within the mouse cortex and that these changes are highly dynamic over the course of neurodevelopment. The opposing direction of changes in excitatory and inhibitory synaptic number suggests that cortical E:I balance is substantially altered in *Chd8*$^{+/-}$ mice, providing a potential mechanism through which loss of function mutations within *CHD8* can manifest as ASD in human patients.

Disclosures: R.A. Ellingford: None. **E. Rabeshala De Meritens:** None. **R. Shaunak:** None. **L. Naybour:** None. **M. Basson:** None. **L.C. Andreae:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.13/A55

Topic: A.07. Developmental Disorders

Support: SFARI
NSERC

Title: Electrophysiological properties of neurons in the primary auditory cortex of the Cntnap2 KO rat model for autism

Authors: *R. S. MANN[1], K. SCOTT[2], S. SCHMID[3];
[1]Western Univ., London, ON, Canada; [2]Anat. and Cell Biol., [3]Univ. of Western Ontario, London, ON, Canada

Abstract: The contactin-associated protein-like 2 (*Cntnap2*) gene, which codes for the cell-adhesion protein CASPR2, is highly important for brain development, especially in sensory structures and areas central for language development. Disruptions in *Cntnap2* can result in neurodevelopment disorder displaying the core symptoms of autism spectrum disorder (ASD) and moderate to severe language impairments in humans. Importantly, the *Cntnap2* gene is not exclusive to humans and is expressed throughout the sensory and cortico-striato-thalamic circuits in other animals. A homozygous *Cntnap2* gene functional knockout in mice and rats is known to disrupt basic functions requiring auditory processing, such as the acoustic startle response and sensorimotor gating. Though it is well established that *Cntnap2* is essential for auditory processing, its mechanisms of action at the cellular level and how it affects neural networks required for auditory processing have not yet been fully characterized. To address this knowledge gap, we used *in vitro* electrophysiology to investigate the changes in membrane properties of neurons within the auditory cortex.
Specifically, the *Cntnap2* gene is hypothesized to be necessary for the maintenance of intrinsic neuronal membrane properties. Neurons in auditory cortex of $Cntnap2^{-/-}$ rats are therefore predicted to have altered intrinsic membrane properties and excitability. Whole-cell patch clamp recordings were performed brain slices from juvenile (postnatal days 8-21) wildtype ($Cntnap2^{+/+}$), heterozygote ($Cntnap2^{+/-}$) and knockout ($Cntnap2^{-/-}$) Sprague Dawley rats. Intrinsic membrane properties, spontaneous miniature EPSCs and IPSCs, firing patterns, and evoked postsynaptic currents of cortical pyramidal cells and PV^{+} fast-spiking interneurons from cortical layers 2/3 were assessed. Sodium and potassium current amplitudes are significantly decreased in both pyramidal neurons and interneurons of *Cntnap2-/-* rats compared to $Cntnap2^{+/+}$ and $Cntnap2^{+/-}$ rats. Preliminary results trend towards smaller action-potential amplitudes and longer half-widths in both pyramidal neurons and interneurons of $Cntnap2^{-/-}$ rats compared to $Cntnap2^{+/+}$. Moreover, the amplitudes and decay times of miniature EPSCs are not different between the genotypes. Preliminary results also indicate that action potential firing rates in response to current injection are not significantly different between the genotypes. These experiments will provide novel insights into how *Cntnap2* impacts auditory processing networks at a cellular level, and shed light on the neural mechanisms underlying altered auditory processing seen in $Cntnap2^{-/-}$ rats.

Disclosures: **R.S. Mann:** None. **K. Scott:** None. **S. Schmid:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.14/A56

Topic: A.07. Developmental Disorders

Title: The contribution of SST-expressing interneurons to the PTEN model of autism spectrum disorder

Authors: *T. W. HOLFORD[1,2], M. BOLTON[1];
[1]Disorders of Neural Circuit Function, Max Planck Florida Inst., Jupiter, FL; [2]Florida Atlantic Univ., Jupiter, FL

Abstract: Autism spectrum disorder (ASD) is a complex disorder with large individual variability, where every case has differences in the type and severity of symptoms. Despite the recent increase in diagnoses, scientists have advanced considerably less in their understanding of the mechanisms of ASD because no individual gene that is implicated in ASD is mutated in more than 1% of patients. One proposed mechanism is that the dysfunction of GABAergic interneurons may play a role in the development and progression of the disorder by interrupting the excitatory and inhibitory balance of neural networks. In our research, we elucidate the role of interneurons in ASD by knocking out one high-risk gene (phosphatase and tensin homologue on chromosome ten, or PTEN) selectively in somatostatin-positive (SST) cells. Since many symptoms of autism spectrum disorder present themselves as social anxieties, we test our mouse model in a variety of settings to observe social interaction and social preference, anxiety-like behavior, and repetitive stereotyped behavior. We found that in the SST-conditional knockout of PTEN, mice had elevated levels of anxiety and fear retrieval, suggesting a potential disruption of amygdala function in these mice. We then investigated potential dysfunction at the cellular and circuit levels using confocal microscopy and electrophysiology. We found that SST-cells lacking PTEN were overgrown morphologically, and they had elevated levels of post-synaptic current following stimulation of neighboring nuclei (from lateral to central amygdala) as well as within the local circuit of the central amygdala. Finally, preliminary evidence suggests that inhibiting central amygdala SST cells in-vivo partially recovers the behavioral deficits observed in the fear and anxiety-related tests, further demonstrating the importance of proper local-circuit function for ASD-related behaviors.

Disclosures: T.W. Holford: None. **M. Bolton:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.15/A57

Topic: A.07. Developmental Disorders

Support: NIH Grant R35N097212
SFARI #401636

Title: Novel phenotypic modifiers cause homeostatic plasticity to fail in five independent *Drosophila* autism models

Authors: *G. W. DAVIS[1], O. GENC[2], J. Y. AN[3], R. D. FETTER[4], Y. KULIK[4], S. J. SANDERS[5];
[1]Biochem. and Biophysics, Univ. California-SF, San Francisco, CA; [2]Biochem. and Biophysics, UCSF, San Francisco, CA; [3]Dept. of Psychiatry, Univ. of California San Francisco, San Francisco, CA; [4]Biochem. and Biophysics, [5]Dept. of Psychiatry, Univ. of California, San Francisco, San Francisco, CA

Abstract: Autism Spectrum Disorder (ASD) is a polygenic disease with no known common underlying pathophysiology. To date, more than 50 gene mutations confer risk for ASD, making it unlikely that therapeutic drug development will proceed without the identification of common underlying pathophysiological processes. We define novel mechanisms that constrain the phenotypic severity of five independent autism gene orthologs: *RIMS1*, *CHD8*, *CHD2*, *WDFY3* and *ASH1L*. First, we analyze heterozygous null mutations in each autism gene for changes in synaptic transmission and presynaptic homeostatic plasticity (PHP). Next, an electrophysiology-based screen and a subsequent systems-genetic analysis of double heterozygous mutant combinations identifies the first known heterozygous mutations that commonly enhance the phenotype of diverse ASD genes, disrupting PHP and neurotransmission. Two modifiers are characterized in detail. Finally, transcriptomic, ultrastructural and electrophysiological analyses define a mechanism for impaired PHP; the maladaptive up-regulation of a novel repressor of PHP. Taken together, we propose the identification of a genetic buffering system that acts to suppress diverse, unrelated ASD gene mutations, and a cascade of negative consequences when this buffering is lost including failure of homeostatic plasticity and cellular robustness.

Disclosures: G.W. Davis: None. **O. Genc:** None. **J.Y. An:** None. **R.D. Fetter:** None. **Y. Kulik:** None. **S.J. Sanders:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.16/A58

Topic: A.07. Developmental Disorders

Support: SFARI Explorer grant
NIH Grant MH096908
Flora Stone Mather ADVANCE Opportunity grant

Title: A GluN2B mutation identified in autism prevents NMDA receptor trafficking and leads to abnormal dendrite growth

Authors: J. BAHRY[1], K. FEDDER[3], Q. WANG[2], M. P. SCENIAK[2], *S. L. SABO[2];
[1]Biochemistry, Cell and Mol. Biol., [2]Biol., Central Michigan Univ., Mount Pleasant, MI; [3]Case Western Reserve Univ., Cleveland, OH

Abstract: Autism spectrum disorders (ASD) are neurodevelopmental disorders with with strong genetic associations. Analysis of *de novo* mutations identified *GRIN2B*, which encodes the GluN2B subunit of NMDA receptors, as a high-probability ASD gene. However, the mechanisms by which *GRIN2B* mutations lead to ASD are not understood. Here, we investigated the cellular phenotypes induced by a human mutation that is predicted to truncate GluN2B within the extracellular loop. This mutation abolished NMDA-dependent calcium influx. Mutant GluN2B co-assembled with GluN1, but the receptor was not trafficked to the cell surface or into dendrites. When mutant GluN2B was expressed in developing cortical neurons, dendrites appeared underdeveloped, with shorter and fewer branches. Mutant dendritic arbors were often dysmorphic, displaying abnormal filopodial-like structures. Interestingly, dendrite maldevelopment appeared when mutant GluN2B was expressed on a wild-type background, reflecting the disease state as ASD individuals are heterozygous for *GRIN2B* mutations. Restoring the fourth transmembrane domain and cytoplasmic tail did not rescue the observed phenotypes. Abnormal dendrite development was not mediated by altered mTOR signaling. Lastly, live-imaging of rat neurons showed that GluN2B mutations altered dendrite growth dynamics. Together, these data support the hypothesis that protein-altering mutations in GRIN2B lead to ASD by disrupting dendrite development.

Disclosures: J. Bahry: None. **K. Fedder:** None. **Q. Wang:** None. **M.P. Sceniak:** None. **S.L. Sabo:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.17/A59

Topic: A.07. Developmental Disorders

Support: R01-MH112694

Title: Investigating synaptic organization using PRobe-based Imaging for Sequential Multiplexing (PRISM)

Authors: *E. W. DANIELSON[1], K. PEREZ DE ARCE[2], E.-C. WAMHOFF[1], J. R. COTTRELL[3], B. CIMINI[3], A. CARPENTER[3], M. BATHE[1];
[1]MIT, Cambridge, MA; [2]Broad Inst., Cambridge, MA; [3]Stanley Ctr. for Psychiatric Res., Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: Genetic studies and molecular analysis of post-mortem human tissue suggest a causal link between synaptic deficits and cognitive disorders such as autism spectrum disorder (ASD), schizophrenia (SCZ), and bipolar disorder (BPD). Despite the strong evidence supporting the importance of a controlled structural assembly of hundreds of synaptic proteins underling synaptic function and electrochemical signaling within neural circuits, simultaneous visualization and *in situ* analysis of several synaptic proteins has been limited by the inability to visualize more than four protein species in any given neuronal sample using conventional imaging approaches. We took advantage of PRISM (**Pr**obe-based **I**maging for **S**equential **M**ultiplexing) confocal imaging approach to achieve high-throughput phenotypic profiling within individual synapses by evaluation of 12 different proteins under network activity inhibition. Quantification analysis of 66-pairwise co-localization patterns from these proteins at inhibitory and excitatory synapses provided an extensive characterization of protein expression profile differences at individual synapses induced by TTX. Using this approach, we found the stoichiometry of PSD-95, SHANK-3 and Homer1b/c was unchanged following TTX, despite increased synaptic levels, indicating coordinated regulation of these proteins. Additionally, we detected a variety of synaptic sub-types, based on the expression-profile, and discovered TTX treatment altered the distribution of synapses within these categories. Building on this work, we use this approach to evaluate undercover effects of genetic perturbation on synaptic proteins profiles. Insight into the remodeling of pre- and post-synaptic protein levels in response to activity blockade and resulting from gene knockdown offers mechanistic insight into the roles of specific gene deletions on synaptic protein organization and neuronal transmission in ASD, SCZ, and BPD.

Disclosures: E.W. Danielson: None. **K. Perez De Arce:** None. **E. Wamhoff:** None. **J.R. Cottrell:** None. **B. Cimini:** None. **A. Carpenter:** None. **M. Bathe:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.18/A60

Topic: A.07. Developmental Disorders

Support: Wellcome Trust
Engineering and Physical Sciences Research Council (EPSRC)

Title: Secreted proteins as modulators of synaptic connectivity and function: A link to autism?

Authors: *F. MCLEOD, G. CLOWRY, A. JACKSON, A. TREVELYAN;
Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Efficient synaptic communication between neurons is fundamental for every brain function. Breakdown in synaptic connections is linked to neurological diseases during development including epilepsy and autism spectrum disorders (ASDs). Therefore, it is crucial to understand the mechanisms that modulate the formation, function and maintenance of synapses in the healthy and diseased brain. Wnt secreted proteins are prominent synaptic modulators in the brain, and de novo mutations in canonical Wnt signalling have been implicated in ASD. To date, the exact contribution of Wnt signalling in ASD has not been characterised. Wnts regulate synapse formation and maintenance, glutamate release and receptor function. Using a combination of confocal microscopy, live imaging and electrophysiological techniques, I have now shown that Wnt7-Fz7 signalling is required for activity-mediated dendritic spine plasticity, AMPA receptor localisation and synaptic strength, all key cellular events involved in learning and memory. Thus, Wnt signalling is essential for the structural and functional plasticity of synapses. Given the integral role that Wnts have at the synapse and their genetic and functional link to ASD, targeted research into the role of Wnts in ASD could provide novel insight into the disease origin. I have developed an optimised system which preserves developing and mature human cortical tissue in culture for days to weeks. Neurons display preserved morphology, neuronal activity and are amenable to viral-mediated transduction. Utilising this technique to study how synaptic structure and function are affected upon regulation of the Wnt pathway could provide a valuable translational model and offer insight into the cellular events leading to neurodevelopmental disorders.

Disclosures: **F. McLeod:** None. **G. Clowry:** None. **A. Jackson:** None. **A. Trevelyan:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.19/A61

Topic: A.07. Developmental Disorders

Title: Isoform-specific roles of ankyrin-B in axonal development and cortical connectivity

Authors: B. A. CREIGHTON, S. AFRIYIE, ***D. N. LORENZO**;
Cell Biol. and Physiol., UNC-Chapel Hill, Chapel Hill, NC

Abstract: Ankyrin-B (AnkB) is an integral component of the membrane-associated cytoskeleton, where it binds the spectrin meshwork to organize specialized membrane domains. AnkB also associates with motor proteins and PI3P lipids on organelles to promotes axonal transport and growth. Two major AnkB isoforms are expressed in the brain, ubiquitously expressed 220kDa (AnkB220) and neuron-specific 440kDa (AnkB440) AnkB. *ANK2*, which encodes AnkB, is a high confidence autism spectrum disorder (ASD) gene and it has been associated with other brain disorders. Loss of both AnkB isoforms in mouse brains (*AnkB*$^{-/-}$) results in absence of long cortical axonal projections and overall reduction in axonal length, confirming that AnkB serves important roles in neuronal development in both humans and mice. In contrast, specific AnkB440 loss increases axonal branching in cultured cortical neurons and *in vivo*. These findings suggest that both AnkB isoforms contribute to axonal development through independent mechanisms that may have a combined effect on cortical connectivity and give rise to functional deficits in ASD patients. To determine the isoform-specific roles of AnkB during axonal development and their role in ASD, we developed mice lacking either AnkB440 or AnkB220 in neural progenitors. We have found that loss of AnkB220 leads to specific deficits in cortical lamination and corpus callosum formation. At the cellular level, we have identified isoform-specific effects on axon growth, morphology, response to axonal guidance cues, and synaptogenesis. Our results indicate that the ankyrin is required for proper signal transduction downstream of guidance receptors to promote actin cytoskeleton remodeling during axon development. We will describe the intracellular mechanisms underlying these functions and how they are affected by ASD-linked AnkB variants.

Disclosures: B.A. Creighton: None. **S. Afriyie:** None. **D.N. Lorenzo:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.20/A62

Topic: A.07. Developmental Disorders

Support: NIH NINDS K08 NS094643
The University of Texas Rising STARs Faculty Award
Child Neurology Society Philip R. Dodge Young Investigator Award
Pediatric Epilepsy Research Foundation (PERF) Scientific Research Grant
Dell Medical School startup funds

Title: Thalamic physiology in the fragile X mouse model of autism

Authors: P. LYUBOSLAVSKY, *A. C. BRUMBACK;
Neurol., Univ. of Texas at Austin, Austin, TX

Abstract: The medial prefrontal cortex (mPFC) and its reciprocal connection with mediodorsal (MD) thalamus are involved in executive functioning and social behavior. Previous work demonstrated that the neurons that provide the main excitatory input from mPFC to MD are hypoexcitable in multiple mouse models of autism including fragile X syndrome model mice. Though there are extensive bodies of work on thalamic physiology in sensory and motor thalamic regions, little is known about the physiology of neurons in the MD thalamus, and particularly those that provide reciprocal inputs to the mPFC. Here, we measured the intrinsic electrophysiological properties of neurons in the three main subdivisions of MD thalamus (medial, central, and lateral MD). To analyze neurons specifically involved in the reciprocal connections between mPFC and MD, we recorded from retrogradely-labeled MD neurons that project to mPFC. We found that MD neurons fell into two categories based on the degree of voltage sag produced in response to hyperpolarizing current steps. We tested the hypothesis that different subtypes of neurons in the three MD subregions would be differentially affected by loss of the fragile X syndrome gene Fmr1. Indeed, we found that the excitability of neurons in MD was not affected uniformly by Fmr1 knockout. Future work will delineate cellular mechanisms for these differences and the role of these specific populations of MD neurons in animal behavior.

Disclosures: P. Lyuboslavsky: None. **A.C. Brumback:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.21/A63

Topic: A.07. Developmental Disorders

Support: NIH Intramural Research Program

Title: Synaptic Kalirin-7 and Trio interactomes reveal a GEF protein-dependent Neuroligin-1 mechanism of action

Authors: *J. PASKUS[1], C. TIAN[2], E. FINGLETON[3], S. MYERS[4], Y. LI[5], B. HERRING[2], K. ROCHE[5];
[1]Natl. Inst. of Hlth., Bethesda, MD, DC; [2]USC, Los Angeles, CA; [3]NINDS, NIH, Bethesda, MD; [4]The Broad Inst. of MIT and Harvard, Cambridge, MA; [5]NINDS, Bethesda, MD

Abstract: The RhoGEFs Kalirin and Trio have emerged as regulators of synaptic plasticity, and their dysregulation has been associated across a variety of neurodevelopmental and neurodegenerative disorders. Although studies have implicated both Kalirin and Trio in certain diseases, such as tauopathies, they remarkably differ in their association with other neurological disorders, namely Autism Spectrum Disorders. We used unbiased proteomic analysis to identify the interactomes of Kalirin-7 and Trio across different developmental timepoints to ascertain distinct protein interaction networks associated with their respective function in synaptic transmission and disease, and revealed groups of proteins that preferentially interact with a particular RhoGEF. We observe a developmental shift in Trio interactions over development, supporting actin and microtubule dynamics early in development. We further show Kalirin-7 is a preferential interactor of the cell adhesion molecule Neuroligin-1 (NLGN1). To determine the functional significance of this interaction, we performed a combination of biochemical, imaging, and electrophysiological experiments showing that NLGN1-dependent synaptic function is mediated through Kalirin-7 in an interaction-dependent manner. Thus, our data reveal not only the first interactomes of two important disease-related proteins, but also provide the first downstream intracellular effector of NLGN1 function.

Disclosures: **J. Paskus:** None. **C. Tian:** None. **E. Fingleton:** None. **S. Myers:** None. **Y. Li:** None. **B. Herring:** None. **K. Roche:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.22/A64

Topic: A.07. Developmental Disorders

Support: NIH Intramural Funding

Title: Functional deficit in NLGN-4Y may contribute to sex bias in NLGN-4X associated autism

Authors: *T. A. NGUYEN[1], M. JAIN[2], A. THURM[3], K. W. ROCHE[4];
[1]NINDS, Bethesda, MD; [2]Kennedy Krieger Inst., Baltimore, MD; [3]Natl. Inst. of Mental Hlth., Bethesda, MD; [4]RBS/NINDS, NIH, Bethesda, MD

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that result in a wide range of behavioral functioning. Interestingly, ASD has long been reported to affect significantly more males than females. The reason for this sex bias in ASDs remains unclear. Among the risk genes associated with ASD, genes on the X chromosome are of particular interest. Neuroligins (NLGNs) are postsynaptic cell adhesion molecules involved in synapse maintenance. There are five NLGNs (NLGN-1, NLGN-2, NLGN-3, NLGN-4X, NLGN-4Y) encoded in the human genome, whereas in rodents there are four (NLGN-1, NLGN-2, NLGN-3, NLGN4-like). NLGN-3 and NLGN-4X are on the X chromosome, and they both have been heavily linked with ASD. NLGN-4X is of interest because it is one of the few genes on the X chromosome that also has a complement gene on the Y chromosome (NLGN-4Y). NLGN-4X and -4Y are remarkably conserved with only 19 amino acid differences between them. Interestingly, many autism-associated mutations have been identified on NLGN-4X, but not NLGN-4Y. Here we show that despite their similarity, NLGN-4X and NLGN-4Y are functionally distinct. Overexpressing NLGN-4Y in heterologous cells or in neurons shows that NLGN-4Y does not traffic efficiently to the cell surface. Chimeras of NLGN-4X and NLGN-4Y demonstrate the lack of surface expression is due to the extracellular domain of NLGN-4Y. Furthermore, we identified a critical region difference between NLGN-4X and -4Y that allows proper protein trafficking for NLGN-4X. Using human genetic data from healthy control and autism databases, we find that autism-associated variants cluster in a critical region. Overexpressing these autism-associated variants in the critical region showed a decrease in surface expression of NLGN-4X similar to that of NLGN-4Y. Taken together, the current data indicate that functional deficits in NLGN-4Y may contribute to the ASD sex-bias, because NLGN-4X mutations will have a dominant effect in males.

Disclosures: T.A. Nguyen: None. **M. Jain:** None. **A. Thurm:** None. **K.W. Roche:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.23/A65

Topic: A.07. Developmental Disorders

Title: Investigation of rare SHANK1 variants found in autistic patients

Authors: *J. JEONG[1], J. S. COHEN[2], C. L. SMITH-HICKS[2], K. W. ROCHE[1];
[1]NINDS, Building 35, Bethesda, MD; [2]Kennedy Krieger Inst., Baltimore, MD

Abstract: The *SHANK* gene family consists of three members (*SHANK1*, *SHANK2*, and *SHANK3*), and each gene encodes a scaffolding protein, which is required for neuronal synapse formation and function. Recent studies have reported the involvement of rare copy-number variations and point mutations on *SHANK* genes in the etiology of autism spectrum disorders (ASD).
We have identified a *de novo SHANK1* frameshift mutation in a patient diagnosed with ASD and a *SHANK1* nonsense mutation in another patient with intellectual disabilities and ASD. Both mutations are *de novo* heterozygous and cause premature termination resulting in truncated Shank1 protein. We hypothesized that the truncated Shank1 proteins play a dominant-negative role and disrupt the normal protein network in dendritic spines, resulting in pathological development of ASD.
Both the frameshift and nonsense *SHANK1* mutants are translated into a truncated form of Shank1 protein in HEK293 cells. We examined the protein interaction of Shank1 mutants with Homer1, a linker protein known to bind to the truncated region of Shank1. The Shank1 mutants showed a complete loss of Homer1 binding in biochemical assays, indicating the mutations can cause severe deficits in binding of Shank1 to its interacting partners and may disrupt protein-protein networks in spines. We further investigated the neuronal localization of Shank1 mutants. Intriguingly, whereas Shank1 WT displayed highly enriched localization in spines, both Shank1 mutants displayed a dispersed localization throughout the spine and dendritic shaft, which indicates impaired synaptic localization of Shank1 mutants.
Improper Shank1 localization and disrupted protein interactions at the synapse caused by the *de novo* mutations likely contribute to the pathological development of ASD. Our future goal is to investigate the functional effect of Shank1 mutants on the development and morphology of dendritic spine and synapse function. Identification of the detailed molecular mechanisms that causes neuronal defects by the SHANK1 mutations would give novel insights into ASD pathophysiology and provide a springboard for molecular therapies.

Disclosures: J. Jeong: None. **J.S. Cohen:** None. **C.L. Smith-Hicks:** None. **K.W. Roche:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.24/A66

Topic: A.07. Developmental Disorders

Title: Glycine transporter type 1 (GlyT1) inhibition improves conspecific-provoked immobility of BALB/c mice: Analysis of cotricosterone response and glucocorticoid expression in cortex and hippocampus

Authors: *J. A. BURKET[1], J. C. PICKLE[1], A. M. RUSK[1], B. A. HAYNES[2], J. A. SHARP[3], S. I. DEUTSCH[1];
[1]Psychiatry and Behavioral Sci., [2]Physiological Sci., [3]Microbiology and Mol. Cell Biol., Eastern Virginia Med. Sch., Norfolk, VA

Abstract: Impaired social communication is a debilitating core symptom domain of autism spectrum disorder (ASD). High levels of comorbid anxiety (~40-50%) are often seen in children and adults with ASD. Recent imaging and physiological studies show altered neurobiological responses to stressors in children with ASD who experience anxiety. Alterations in stress reactivity and glucocorticoid signaling have been documented in ASD patients, as well as relevant mouse models of ASD, with anxiety-like behaviors observed in several well-characterized models. D-Cycloserine, a partial glycine$_B$ agonist of the NMDA receptor (NMDAR), and VU0410120 (VU120), a novel glycine type-1 transporter inhibitor, improved sociability in Balb/c mice, suggesting that NMDAR activation regulates sociability, and the endogenous tone of NMDAR-mediated neurotransmission is altered in this strain. Conspecific-provoked immobility (CPI) of Balb/c mice in the presence of enclosed and freely-behaving stimulus mice was investigated using a 3-chambered sociability apparatus. We explored the relationship between CPI and serum corticosterone (CORT) response and determined effects of a prosocial dose of VU120 in Balb/c mice on these measures. On all measures, Balb/c mice were more immobile than comparator Swiss Webster (SW) mice ($p<0.05$). There were no differences in CORT levels between vehicle-treated Balb/c and SW mice or between vehicle-treated Balb/c mice with high (>73s; N=8) or low (<73s; N=9) immobility scores. VU120-treated Balb/c mice (N=21) showed significantly less immobility and higher CORT levels compared to vehicle-treated mice. Regardless of high (N=3) or low (N=8) immobility, CORT levels of VU120-treated Balb/c mice (total N=11) did not differ. Finally, we conducted a targeted comparison of gene expression profiles of 88 glucocorticoid signaling associated genes within frontal cortex and hippocampus. Effects of VU120 treatment and differences between SW and Balb/c mouse strains and their prosocial response categories were determined by fold-change ($-1.25 <$ or > 1.25, $p \leq 0.05$; FDR $p \leq 0.05$). Results from these analyses show that expression of the gene encoding Ddit4, a known negative regulator of mTORC1 signaling, was significantly increased within

frontal cortex in VU120-treated Balb/c “non-responders”, compared to both VU120-treated Balb/c “responders” and the VU120-treated SW “medium” social response group. In conclusion, the prosocial effect of VU120 was unrelated to conspecific-provoked CORT response in Balb/c mice. Social stress alone may not determine increased immobility in Balb/c mice; this strain may also display an element of social disinterest.

Disclosures: **J.A. Burket:** None. **J.C. Pickle:** None. **A.M. Rusk:** None. **B.A. Haynes:** None. **J.A. Sharp:** None. **S.I. Deutsch:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.25/A67

Topic: A.07. Developmental Disorders

Support: Simons Foundation SFARI Award 573689

Title: Foxp1 regulates dopamine-receptor 2 striatal neuron excitability through changes in subthreshold potassium currents

Authors: *N. KHANDELWAL, V. RYBALCHENKO, S. CAVALIER, A. G. ANDERSON, G. KONOPKA, J. R. GIBSON;
Basic Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: The suppressed function of transcription factor Foxp1 is strongly linked with autism. In an earlier study we have reported that dopamine receptor 2 expressing (D2) striatal projection neurons (SPNs) have higher intrinsic excitability in *Foxp1*$^{+/-}$ mice (Araujo et al., 2015). However, the cause for this hyper-excitability is not yet known. To understand the underlying mechanisms, we examined the SPNs in mice where only one subset of SPNs underwent homozygous *Foxp1* deletion. In mice where *Foxp1* was deleted selectively in D2 SPNs, these neurons displayed strikingly more hyper-excitability compared to that observed in *Foxp1*$^{+/-}$ mice. The most salient changes were observed in two of the subthreshold properties of the neurons: an increase in input resistance and a more depolarized resting membrane potential. We believe these effects are due to the decrease in two types of potassium (K^+) currents i.e. inwardly rectifying and leak currents. There were no differences in the size of soma or dendritic tree which suggests that the hyper-excitability is caused mainly due to the changes in the ion channel function. Moreover, these effects are significantly more prominent when *Foxp1* is deleted embryonically as compare to the postnatal deletion. With selective homozygous *Foxp1* deletion in the dopamine-receptor 1 (D1) SPNs, these neurons also had increased intrinsic excitability. However, the effect was not as strong as that observed for D2 SPNs, and subthreshold membrane properties were not detectably altered. Our results suggest that Foxp1 negatively regulates

excitability in both of the SPNs, however the mechanism for this regulation is different in both subtypes.

Disclosures: **N. Khandelwal:** None. **V. Rybalchenko:** None. **S. Cavalier:** None. **A.G. Anderson:** None. **G. Konopka:** None. **J.R. Gibson:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.26/A68

Topic: A.07. Developmental Disorders

Support: NIH/NINDS grant #R37 NS-071785-07

Title: Hippocampal network dysfunction and interneuron-based cell therapy in null Cntnap2 mouse model of autism spectrum disorder

Authors: *R. PATERNO[1], J. R. MARAFIGA[2], S. C. BARABAN[3];
[1]Epilepsy Res. Lab. and Weill Inst., San Francisco, CA; [2]Grad. Program in Biol. Sciences: Biochemistry, Dept. of Biochemistry, ICBS, Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil; [3]Dept Neurolog Surgery, Univ. California San Francisco, San Francisco, CA

Abstract: Autism spectrum disorder (ASD) is a significant neurodevelopmental condition characterized by impairment in social interactions, hyperactivity, and repetitive actions. The underlying pathophysiological mechanism is characterized by impaired GABAergic neurotransmission associated with a decreased number of PV+ interneurons in different brain regions including the hippocampus. Despite this known neurological basis of ASD, there are no therapies available to completely rescue behavioral deficits. Because our recent work has shown that interneuron-based cell therapy and specifically transplanting embryonic GABA progenitors derived from medial ganglionic eminence (MGE) can rescue neuronal network imbalance and animal behavior in animal models of epilepsy, we began to explore whether this treatment can also be therapeutic in ASD. We used an animal model of autism (contactin-associated protein-like 2 knockout - *Cntnap2* KO) to assess behavioral phenotype(s), identify hippocampal neural network changes and test whether intra-hippocampal MGE transplantation is therapeutic. In a series of behavioral assays, we assessed social interaction, anxiety level, locomotor activity, repetitive behavior and episodic-like memory in *Cntnap2* KO mice and age-matched WT sibling controls. We used 32-channel silicon probes arrayed across CA1-to-DG axis to record *in vivo* local field potential signals while mice navigated a novel/familiar paradigm task. Hippocampal oscillations were analyzed using custom-written MATLAB-based software. In an additional cohort of mice, we transplanted embryonic MGE cells into the hippocampus at postnatal day 2. Here, we present three major findings: (1) *Cntnap2* KO mice showed impaired social interaction

and repetitive behavior manifest as higher level of self-grooming compared to WT as well as hyperactivity during open field performance; (2) complex disruption of theta-gamma oscillations along the CA1-to-DG axis; (3) migration, differentiation and functional integration of MGE cells. These results highlight the complex behavioral and network issues associated with ASD, and suggest a potential therapy based on the integration of new inhibitory neurons.

Disclosures: R. Paterno: None. **J.R. Marafiga:** None. **S.C. Baraban:** None.

Poster

030. Autism: Synaptic and Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 030.27/A69

Topic: A.07. Developmental Disorders

Support: NRF Grant by the Korean Government 800-20190161

Title: Striatal transcriptomic analysis reveals the role of gene x in synaptic and behavioral deficits of ASD mouse model

Authors: *H. KIM, H.-S. KIM;
Dept. of Pharmacol., Seoul Natl. Univ. Col. Med., Seoul, Korea, Republic of

Abstract: The striatum of the basal ganglia is the major subcortical component of the mammalian forebrain. MRI studies have implicated surface deformation of striatum in the brains of patients with Autism spectrum disorder (ASD) and their correlation with behavioral phenotypes. In the present study, we analyzed transcriptome alteration using RNA sequencing (RNA-Seq) with striatal tissues from 10-week-old ASD mouse model. Expression levels of differentially expressed genes obtained through RNA-Seq were confirmed using qPCR and western blot analysis. Expression of Gene X was significantly upregulated in the striatal tissues from the ASD mouse model. In addition, it was found that Gene X is expressed in neurons as well as microglia, and the spine density of neurons was decreased in dorsomedial striatum of ASD mouse model, compared with control group. Medium spiny neurons in the striatum are divided to 2 types by which dopamine receptor those express. The expression of dopamine receptor 2 (D2) was investigated and found to be increased in striatum of ASD mouse model. These results suggest that increase in Gene X expression is a mechanism related to the change in synaptic plasticity and behavioral phenotypes of ASD mouse model.

Disclosures: H. Kim: A. Employment/Salary (full or part-time):; Department of Pharmacology, College of Medicine, Seoul National University, 103 Daehakro, Jongro-gu, Seoul, Republic of Korea, Department of Biomedical Sciences, College of Medicine, Seoul National University, 103 Daehakro, Jongro-gu, Seoul, Republic of Korea. **H. Kim:** A.

Employment/Salary (full or part-time):; Department of Pharmacology, College of Medicine, Seoul National University, 103 Daehakro, Jongro-gu, Seoul, Republic of Korea, Department of Biomedical Sciences, College of Medicine, Seoul National University, 103 Daehakro, Jongro-gu, Seoul, Republic of Korea.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.01/A70

Topic: A.09. Adolescent Development

Support: Regione Sardegna GRANT CRP-60921

Title: Social isolation induced deficits of neuronal plasticity: Reversal by social housing

Authors: *M. C. MOSTALLINO[1], F. BIGGIO[2], G. TALANI[1], V. LOCCI[3], R. MOSTALLINO[2], E. SANNA[2,1], G. BIGGIO[2,1];
[1]Inst. of Neurosci., Natl. Res. Council, CNR, Cagliari, Italy; [2]Dept of Life and Envrn. Sci., Univ. of Cagliari, Cagliari, Italy; [3]Dept. of Psychiatry, Univ. of Illinois, Westchester, IL

Abstract: Deprivation of social contact in rats during adolescence is a chronic stress that leads to long-lasting alterations in their behavioural profile. Social isolation (SI) results in changes of emotional state, abnormal reactivity to environmental stimuli that are associated to changes in brain plasticity and hormonal secretion such as reduction in the brain and plasma concentrations of progesterone and its metabolites 3α,5α-TH PROG and 3α,5α-TH DOC, two neurosteroids that modulate neuronal excitability and plasticity. On the contrary environmental enrichment is know to improve brain plasticity and protect synaptic function from negative insults.
In the present study we used the exposure to social enrichment (SE) in order to ameliorate the negative effect observed in post weaning isolated male rats in which neurotrophic factors [Brain-Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF)], neurogenesis, neuronal dendritic trees and spines were markedly reduced in the hippocampus (Hipp).
The amount of BDNF and NGF proteins were significantly decreased in whole Hipp of SI male rats from postnatal day 21 for 8 weeks (1 for cage) compared with rats housed in standard conditions (6 for cage). In agreement with the putative role of BDNF and NGF in the regulation of synaptic plasticity, the changes in Arc protein (Activity-Regulated Cytoskeletal Protein is as a marker for plastic changes in the brain) and in density and morphology of dendritic spines, as well as in neuronal tree arborization of granule cells in the dentate gyrus were similar to those observed for the BDNF and NGF proteins in Hipp. All these changes were associated with a marked decrease in neuronal proliferation and neurogenesis in the dentate gyrus of Hipp formation. Moreover, all these parameters were totally restored in the rats isolated for 4 weeks followed by 4 weeks of reunion.

Recent evidences suggest that synaptic consolidation and neurogenesis requires BDNF and NGF signaling and induction of Arc protein. By modulating the translation of newly induced Arc protein in dendrites, BDNF and NGF may control the window of synaptic consolidation and trophism of dendritic tree. Dysregulation of BDNF, NGF, Arc synthesis, density and morphology of dendritic spines they might be important determinants in the functional regulation of those synapses and neurogenesis, might play a crucial role in the modulation of the emotional and affective behaviours.
These data further suggest that exposure to SE, abolishes the negative effect of SI stress on Hipp plasticity, an effect that might improve neuronal resilience with a beneficial effect on cognitive function.

Disclosures: M.C. Mostallino: None. **F. Biggio:** None. **G. Talani:** None. **V. Locci:** None. **R. Mostallino:** None. **E. Sanna:** None. **G. Biggio:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.02/A71

Topic: A.09. Adolescent Development

Title: Perinatal dietary omega-3 fatty acid deficiency reduces maternal nurturing and adversely impacts postnatal brain development in rats

Authors: *R. H. ASCH, J. D. SCHURDAK, R. K. MCNAMARA;
Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Having a biological parent with a mood disorder substantially increases the offspring's risk of developing a mood disorder, and this familial transmission is believed to involve both genetic and environmental factors. One candidate environmental risk factor is a dietary deficiency in essential nutrients, including omega-3 (*n*-3) fatty acids. Docosahexaenoic acid (DHA) is the most abundant *n*-3 in brain and rapidly accrues in the developing prefrontal cortex (PFC) in parallel with rapid and enduing maturational changes. Notably, individuals with mood disorders, including females of childbearing potential, exhibit robust *n*-3 deficits. This study used an animal model to test the hypothesis that preconception *n*-3 deficiency influences maternal nurturing behavior and postnatal developmental trajectories of the offspring. Adult female rats received a control diet (CON) containing the essential *n*-3 alpha-linolenic acid or an *n*-3 deficient diet (DEF) beginning 30 days prior to mating until postnatal day 21 (P21), at which point pups were weaned onto the same diet as their dams. Dam-offspring interactions were video recorded on P3, P6, and P9, and maternal behaviors scored by a trained rater blinded to diet. Molecular markers of PFC maturation were assessed in P21 offspring using a qRT-PCR gene array. Locomotor response to acute and chronic amphetamine (1 mg/kg) was evaluated in

adolescents (P40-P80), and fear conditioning was conducted in young adult offspring (P90-92). PFC and blood fatty acid levels were measured by gas chromatography. DEF dams exhibited lower blood and PFC DHA levels compared with CON dams, and spent significantly less time engaged in nurturing behaviors including arch-back nursing and pup licking and grooming. DEF neonates exhibited lower PFC DHA levels and at P21 had gene expression profiles consistent with delayed PFC maturation, including reduced myelin-basic protein and elevated GAP-43 expression. DEF adolescents had a blunted locomotor response to acute amphetamine, which normalized following chronic treatment, and exhibited impaired fear extinction as young adults. Together these results demonstrate that perinatal *n*-3 deficiency reduces maternal nurturing behavior and alters offspring PFC development, responsivity to amphetamine, and fear learning and memory. These preclinical data provide proof-of-concept evidence that perinatal *n*-3 deficiency can cause enduring developmental abnormalities in offspring that may be relevant to the etiology of emotional dysregulation associated with mood disorders.

Disclosures: R.H. Asch: None. **J.D. Schurdak:** None. **R.K. McNamara:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.03/A72

Topic: A.09. Adolescent Development

Title: Smartphone abstinence increases students' daily physical activity and sleep: A controlled interventional trial

Authors: *M. SPITZER[1], S. LORENZ[1], K. WIEDENHORN-MÜLLER[1], A. BERGER[1], T. KAMMER[2];
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Abstract: The smartphone has become the globally most widely distributed digital device and it is most used in terms of hours per day. In order to investigate its effects on well-being and daily living in students, we studied students who volunteered to forego their use of digital media, including their smartphone. In a prospective randomized controlled trial with a within subject cross-over design, 206 students of classes 6 to 10 of a German secondary school (Gymnasium) at age 11-17 stopped using their smartphone and other digital screen media for 4 weeks. Screen media abstinence was implemented in the entire class, with randomization between classes of the same age. There were two abstinence-periods of 4 weeks each, with measurements and tests before and at the end of each period. Half of the classes (n=105) were abstinent during the first period, while the other half (n=101) was abstinent during the second period. Dependent variables were media-use (according to daily protocols), as well as physical activity and sleep duration by

activity tracking wristbands. In the control (non-abstinence) periods students reported daily screen time of 2h32 min. The abstinence intervention reduced mean daily screen time to 12 min. Students walked about 8400 steps per day. Smartphone abstinence increased physical activity by 852 steps per day (99% CI 333 - 1389 steps/d), i.e., by about 10%. Mean sleep duration was 8h33min, smartphone abstinence increased duration by 8.4 minutes (99% CI 0.6 - 16.4 min). Our measurements are in line with reported physical activity levels (measured as steps per day) in other countries (USA, Canada) and also reproduced the findings of fewer steps in girls compared to boys, as well as a slight decrease of daily steps in adolescence every year. To our knowledge this is the first prospective experimental study under real live conditions on the effects of smartphone use on physical activity in adolescents. It adds to the growing body of evidence on the detrimental effects of smartphones on health which so far is mainly correlational. Based upon the finding that a 10% reduction of physical inactivity globally could avert 533 000 deaths per year (Lee et al. 2012, Lancet 380:219) the detrimental effects of digital IT use in general, and smartphone use by more than two thirds of the world's population in particular, on physical inactivity, and hence on life expectancy, can be estimated to be in the same range.

Disclosures: **M. Spitzer:** None. **S. Lorenz:** None. **K. Wiedenhorn-Müller:** None. **A. Berger:** None. **T. Kammer:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.04/A73

Topic: A.09. Adolescent Development

Title: Adolescent obesity specifically alters stress coping in adulthood without changes to working memory or anxiety

Authors: *K. R. LLOYD, T. M. REYES;
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Abstract: Adolescent obesity is an important clinical problem, with prevalence at approximately 20%. Adolescence is a time of rapid maturation of the prefrontal cortex, so disruptions to this maturation could have lifelong consequences. Most of the high-fat diet rodent literature involves long feeding periods and performing behavioral testing during diet exposure. We created a temporally-restricted model that limited high fat diet feeding to the adolescent period, allowing us to study this developmental period specifically and avoid the confound of different diets during behavior testing. Using this model we have previously found a male-specific motivational deficit and a female-specific increase in learning and decrease in inattention during operant testing. In this experiment we examined other PFC-dependent behaviors using a variety of tasks with fewer training demands than operant testing. C57/BL6/J x DBA F1 hybrid mice were fed

either a high fat diet (HFD, 60% calories from fat) or a standard fat control diet (20% calories from fat) from weaning to 7 weeks of age, when all animals were switched to standard laboratory chow. Carcass NMR was used to determine fat and lean mass in animals sacrificed one day after the diet switch. We found that HFD fed animals were heavier and had greater percent body fat than controls. Another group of animals underwent a variety of behavioral tests between 7 and 13 weeks of age to investigate prefrontal cortex function. No differences were found in the novel object recognition task or sugar or fat preference. There were no diet effects on anxiety, but females tended to be less anxious, spending more time in the center in the open field task and making more head dips and stretch attend postures in the elevated zero maze. There were no differences on measures of locomotor activity. A diet effect was seen in the forced swim test, with HFD-fed animals spending more time immobile and less time climbing than controls. This difference cannot be explained by differences in buoyancy or locomotor activity, as the two groups did not differ on bodyweight, body length, or velocity and distance traveled in the open field. We conclude that our adolescent HFD paradigm induces obesity that resolves in adulthood and specifically increases passive coping behavior in response to acute stressors.

Disclosures: K.R. Lloyd: None. **T.M. Reyes:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.05/A74

Topic: A.09. Adolescent Development

Support: NIH R01 MH101533 (S.S.D.)

Title: Racial and sex disparities in newborn telomere length are predicted by maternal early life adversity exposure

Authors: *L. W. Y. MCLESTER-DAVIS, C. W. JONES, K. C. ESTEVES, S. S. DRURY; Tulane Univ., New Orleans, LA

Abstract: BACKGROUND: The Adverse Childhood Experiences (ACEs) survey is a self-report measure that captures a range of negative early life events and is predictive of multiple future health risks within the individual. Furthermore, greater ACE exposure is associated with poor birth outcomes suggesting risks may span generations. One possible pathway through which ACE exposure is transmitted across generations is through telomere dynamics. Telomere length (TL) is a marker of cellular aging; shorter TL is associated with negative health outcomes and early life adversity. Newborn TL is a predictor of TL attrition across the life-course and is associated with poor birth outcomes, as such newborn TL may capture both maternal exposure and predict future health risk. **OBJECTIVES:** To examine the impact of maternal ACE score on

newborn TL and investigate how race and sex moderate this relation. **METHODS:** N=336 pregnant women provided information on ACE exposures, prenatal stress, SES, pregnancy complications, and demographics prenatally. DNA was extracted from newborn bloodspots for TL analyses and evaluated for double-stranded DNA integrity and concentration. The average relative TL was determined through the use of duplicate plates with each sample in triplicate by using an adapted MMQ-PCR. TL was estimated as the ratio (T/S) of the telomere repeat to a single gene (albumin) copy number. Descriptive statistics characterized the sample overall, and among blacks and whites separately. Generalized linear regression models tested the impact of maternal ACE on newborn TL and examined the moderation by race and sex. All models were adjusted for prenatal stress, SES, and pregnancy complications. **RESULTS:** Black newborn TL was significantly longer than white (β=-0.108, 95% CI[-0.190, -0.027]; P=0.009). Consistent with our previous results, black females had significantly longer TL than other groups. Maternal ACE was not associated with newborn TL. However, race-stratified analyses revealed a significant association in white newborns (β=-0.033, 95% CI[-0.060, -0.005]; P=0.021, n=127) but no relation in black newborns (β=0.005, 95% CI[-0.018, 0.028]; P=0.681, n=209). **CONCLUSIONS:** Racial differences in TL were already present at birth, with black female infants exhibiting the longest TL. Maternal ACE score predicted shorter TL only in white newborns. These findings suggest that, at birth, racial and sex differences in the biological vulnerability to maternal preconception adversity already exist.

Disclosures: L.W.Y. McLester-Davis: None. **C.W. Jones:** None. **K.C. Esteves:** None. **S.S. Drury:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.06/A75

Topic: A.09. Adolescent Development

Support: DGAPA-PAPIIT IA205218
DGAPA-PAPIIT IN215218
DGAPA-PAPIIT IN217918

Title: CB2R regulate self-control in adolescents, adults and aged Wistar rats

Authors: *D. A. RANGEL RANGEL[1], Y. A. ALVARADO RAMÍREZ[4], L. A. BECERRIL MELENDEZ[2], A. E. RUIZ-CONTRERAS[6], O. PROSPERO-GARCIA[3], M. MENDEZ DIAZ[5]; [1]Fisiología, [2]UNAM, México, Mexico; [3]UNAM, Mexico, D. F., Mexico; [4]Univ. Nacional Autonoma De Mexico, Ciudad de Mexico, Mexico; [5]Univ. Nacional Autonoma De Mexico, Mexico DF, Mexico; [6]Lab. Neurogenomica Cognitiva, Fac. Psicologia, UNAM, D.F., Mexico

Abstract: Adolescents make more errors in decision making than adults, exhibiting self-control deficiency thereby risky behavior, i.e. use of drugs of abuse. This condition may be due to an immature behavior control system (BCS) that depends in part on prefrontal cortex function that control subcortical structures, i.e. nucleus accumbens. On the other hand, cannabinoid receptor 2 (CB2R) selective activation decreases intravenous cocaine self-administration, suggesting CB2R participates in the BCS. The goal of this study was to determine if CB2R is involved in the BCS. To reach this goal we evaluated impulsivity using 5-CSRTT in adolescents (PND 28-45), adults (PND 90-120) and aged (PND 365) rats. We also estimated by immunofluorescence, the expression of CB2R in prefrontal cortex (PFC), nucleus accumbens (NAc) and lateral habenula (LHb). Results. Adolescent rats show a greater number of impulsive responses compared to adults. The aged rats failed to reach the training criteria. CB2R seems to be differentially expressed in PFC, NAc and LHb in adolescent rats compare adults and aged rats suggesting its participation in the BCS.

Disclosures: D.A. Rangel Rangel: None. **Y.A. Alvarado Ramírez:** None. **L.A. Becerril Melendez:** None. **A.E. Ruiz-Contreras:** None. **O. Prospero-Garcia:** None. **M. Mendez Diaz:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.07/A76

Topic: A.09. Adolescent Development

Support: U01DA041174

Title: Behavioral and neural predictors of vulnerability for risky behaviors in childhood

Authors: *K. M. RAPUANO[1], M. D. ROSENBERG[1], C. HORIEN[2], A. S. GREENE[2], D. SCHEINOST[3], R. T. CONSTABLE[3], B. J. CASEY[1];
[1]Dept. oF Psychology, [2]Yale Sch. of Med., [3]Dept. of Radiology and Biomed. Imaging, Yale Univ., New Haven, CT

Abstract: Risky behaviors such as substance abuse increase during adolescence; however, neurodevelopmental associations underlying vulnerability to substance use remain less well understood. Here, we sought to develop behavioral and neural models of substance use vulnerability in childhood. Responses to substance use-related questions in 11,875 nine- and ten-year-old children participating in the Adolescent Brain and Cognitive Development (ABCD) study (Casey et al., 2018; Lisdahl et al., 2018) were used to characterize a behavioral indicator of substance use vulnerability. A principal components analysis of parent and child responses revealed two orthogonal components that loaded highly on child knowledge of and intention to

use substances (i.e., PC1) and familial factors related to substance use (i.e., PC2). Component loadings were validated across twenty-one sites to determine the reliability of dimensions associated with risk. These behavioral components were used to generate connectome-based predictive models (CPM; Shen et al., 2017) of vulnerability based on resting-state functional brain connectivity. Individual differences in PC1 scores were significantly predicted in left-out subjects using CPM; however, neural models were not predictive of PC2 scores. These findings suggest that the developing brain reflects differences in substance use-related risk factors and that these differences may be predicted prior to substance use initiation. Further, these results imply that risk factors associated with child intent are distinguishable from risk factors associated with family history of abuse, which may emerge later in development. These findings set the groundwork for future prediction of substance use initiation and chronicity in adolescents.

Disclosures: K.M. Rapuano: None. **M.D. Rosenberg:** None. **C. Horien:** None. **A.S. Greene:** None. **D. Scheinost:** None. **R.T. Constable:** None. **B.J. Casey:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.08/A77

Topic: A.09. Adolescent Development

Support: UIUC RB 17146
APF/COGDOP Peter and Malina James & Dr. Louis P. James Legacy Scholarship

Title: Role of GluN2B function in extinction and reinstatement of methamphetamine self-administration in adolescent and adult rats of both sexes

Authors: *S. R. WESTBROOK[1], E. R. CARLSON[1], J. P. O'RUSSA[1], K. A. HAMBLEN[1], J. M. GULLEY[1,2];
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Abstract: Previous studies in rodents suggest that adolescents are more resistant to extinction compared to adults. Although the neural mechanisms underlying this age difference are currently unknown, studies in adult rats suggest a role for GluN2B-containing NMDA receptor function in the consolidation of extinction memory. Importantly, GluN2B function emerges during adolescent development. Drugs of abuse may delay the ontogeny of extinction consolidation through altering the developmental trajectory of GluN2B neurotransmission, which may contribute to age-of-onset differences in extinction and subsequent reinstatement of drug-seeking behaviors. Here, we investigated this hypothesis as outlined in our pre-registration on the Open Science Framework (https://osf.io/avkhf) by training male (n=42) and female (n=44) Sprague-

Dawley rats to self-administer methamphetamine (METH, 0.1 mg/kg/infusion i.v.) starting during adolescence or adulthood (41 or 91 days old, respectively). Rats were allowed to self-administer METH under 2-h short access (ShA) conditions for seven days and 6-h long access (LgA) conditions for the following 14 days. Subsequently, rats underwent four daily 30-min extinction sessions with immediate post-session injections of either a GluN2B antagonist (Ro25-6981; 6 mg/kg, i.p.) or a vehicle solution. Over the next four days, rats received four 2-h extinction sessions. Finally, rats received a priming injection (1 mg/kg METH, i.p.) 30 min before a 2-h reinstatement session. We found that all groups escalated their METH intake across LgA sessions, with adolescent-onset rats of both sexes having higher METH intake than their adult-onset counterparts. All groups reduced their responding in the previously reinforced nosepoke port across extinction sessions; however, we found no evidence for significant effects of age-of-onset, sex, or GluN2B antagonism on extinction consolidation. All groups reinstated drug-seeking behavior in response to the METH priming injection, with adult-onset males reinstating the least. These results do not support our hypothesis that adolescent-onset METH use would disrupt the functional emergence of GluN2B transmission and contribute to age-of-onset differences in extinction of METH-seeking. However, our findings suggest that adolescent-onset METH use may lead to greater drug intake. Furthermore, our reinstatement data support the view that age-of-onset and sex are factors that contribute vulnerability to relapse to METH-seeking.

Disclosures: S.R. Westbrook: None. **E.R. Carlson:** None. **J.P. O'Russa:** None. **K.A. Hamblen:** None. **J.M. Gulley:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.09/A78

Topic: H.01. Animal Cognition and Behavior

Support: CNPq EDITAL UNIVERSAL 2016
CAPES
UFRGS

Title: Maternal deprivation as a model of early-life stress alters maternal behavior, olfactory learning and neural development

Authors: D. CZARNABAY[1,2], J. DALMAGO[1], A. S. MARTINS[1], A. QUEIROZ[1], L.-E. SPERLING[1,3], K. REIS[1,3], P. PRANKE[1,3], ***F. BENETTI**[4,2];
[1]Physiol., Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil; [2]Inst. of Basic and Hlth. Sci., Basic Physiol. and Cognition Lab., Porto Alegre, Brazil; [3]Inst. de Pesquisas com Células-Tronco, Hematology and Stem Cell Lab., Porto Alegre, Brazil; [4]Physiol., Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil

Abstract: Early life stress such as physical abuse, trauma or neglect during a critical period of development can elicit negative long-lasting effects on health. Neonatal maternal deprivation (MD) is a stressful event capable of triggering structural and neurobiological changes in Central Nervous System (CNS) development during proliferative and migratory cell differentiation. In this study, we investigated the maternal behavior of lactating rats submitted to protocol of chronic neonatal maternal deprivation (MD) during postnatal day (PND) 1 to 10. We analyzed the effects of the MD in the olfactory memory and cellular proliferation and differentiation in the hippocampus and olfactory bulb in *Wistar* rat pups on 7, 11 and 21 days postpartum. Analysis in active neurons, cellular differentiation and proliferation, were marked and evaluated by flow cytometry in tissue samples of hippocampi and olfactory bulb. Our results demonstrated an increase in maternal behavior immediately after dam's return to the home-cage in MD group compared to the non-deprived group. In addition, MD pups spent more time (higher latency) to identify the nest odor in comparison to the non-deprived rat pups in the olfactory learning task and showed a significant delay in the neural differentiation and proliferation in the hippocampus and olfactory bulb. These results reveal that disruptions in the mother-infant bonding by the MD induce changes in maternal behavior and interaction with the offspring that could be leading to delayed CNS development and significant impairment in offspring's olfactory learning.

Disclosures: D. Czarnabay: None. **J. Dalmago:** None. **A.S. Martins:** None. **A. Queiroz:** None. **L. Sperling:** None. **K. Reis:** None. **P. Pranke:** None. **F. Benetti:** None.

Poster

031. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 031.10/A79

Topic: H.01. Animal Cognition and Behavior

Support: FONCICYT-DADC 273553
Conacyt CB250880
DGAPA PAPIIT IN210817
Conacyt CB253222

Title: Increasing dopaminergic activity improves synaptic plasticity and memory performance in cognitively impaired animals due to chronic exposure to a high-sugar diet

Authors: *E. S. GUTIERREZ-LOPEZ[1], L. F. RODRÍGUEZ[1], S. HERNÁNDEZ[2], D. OSORIO-GÓMEZ[1], P. SALCEDO-TELLO[1], M. VELASCO[1], M. HIRIART[1], F. BERMÚDEZ-RATTONI[1], K. GUZMAN-RAMOS[3];
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Abstract: Several studies have shown that overweight and obesity increase the risk of suffering metabolic and cognitive dysfunctions. The excessive accumulation of body fat, generally due to an imbalance between the calories consumed and spent, produces the metabolic syndrome, which is considered a risk factor for the development of cognitive impairment. The mechanisms underlying this cognitive decline are unknown. However, several studies indicate that chronic exposure to high-calorie diets affects the dopaminergic system, a neuromodulator for the formation of declarative memory and neuronal plasticity. Therefore, a chronic diet with high sugar content could dysregulate dopaminergic activity in the hippocampus, causing deterioration in the synaptic plasticity underlying spatial memory. To test this hypothesis, we measured release of neurotransmitters in the dorsal hippocampus by *in vivo* microdialysis during an object location recognition memory task. In addition, neuronal plasticity was evaluated by long-term potentiation in the perforating to dentate gyrus pathway of male Wistar rats. A group of rats were exposed to a 20% sucrose solution for 6 months and compared to control group of rats exposed to tap water for the same number of months. The high-sucrose diet caused an increased in adiposity, glucose intolerance, insulin resistance and dyslipidemia; constituting a model of metabolic syndrome. The behavioral results indicate an impairment of the spatial memory and a deficiency in the synaptic strength in rats with metabolic syndrome, which was correlated with a decrease in the dopaminergic release during the object location recognition memory. In addition, intra-hippocampal treatment with nomifensine in rats with metabolic syndrome produced an increase in synaptic strength similar to controls. Likewise, rats with metabolic syndrome treated with nomifensine behaved similarly to controls in spatial recognition memory tests. These results show that the restoration of dopaminergic activity can reverse the deterioration of recognition memory and long-term plasticity. These results will help to understand the mechanisms of cognitive deterioration in the metabolic syndrome and how the deterioration of the memory of this syndrome could be reversed.

Disclosures: E.S. Gutierrez-Lopez: None. **L.F. Rodríguez:** None. **S. Hernández:** None. **D. Osorio-Gómez:** None. **P. Salcedo-Tello:** None. **M. Velasco:** None. **M. Hiriart:** None. **F. Bermúdez-Rattoni:** None. **K. Guzman-Ramos:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.01/A80

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Foundation for Geriatric Diseases at KI 2018-01292
Gun & Bertil Stohnes Foundation 2019
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Title: Functional remodeling of excitatory synapses in the hippocampus induced by astrocyte glutamate transporters dysfunction

Authors: *E. VAZQUEZ-JUAREZ, M. LINDSKOG;
Karolinska Institutet, Stockholm, Sweden

Abstract: At excitatory synapses, glutamate uptake is carried out by glutamate transporters predominantly expressed in astrocytes and play an important role in shaping the spatiotemporal profile of neurotransmission. Since diverse studies have pointed out the decrease in expression of glutamate transporters in psychiatric and neurodegenerative diseases, we have addressed the correlation between glutamate transport impairment and synaptic alterations observed in pathological conditions. To do so, we have monitored glutamate levels and evoked synaptic responses from CA1 neurons in hippocampal slices during acute inhibition of glutamate transporters by DL-TBOA. To our surprise, although DL-TBOA leads to a more that two-fold increase in extracellular glutamate levels, there is a gradual reduction in the field EPSPs. This long-lasting decrease in synaptic strength can be mimicked by bath application of glutamate and involves NMDA receptors since it can be prevented by MK-801. A significant feature of the synaptic adaptation to DL-TBOA is a partial recovery of the response even in the presence of the blocker, reaching a new plateau of stable EPSP amplitude that it's only slightly increased after wash-out. Interestingly, this partial recovery of the synaptic response depends on constant presynaptic stimulation and we have found that exogenous application of BDNF, a known powerful activity-dependent synaptic modulator, can also partially restore synaptic response in DL-TBOA-treated slices in the absence of constant presynaptic stimulation.
To address the impact of the DL-TBOA-induced long-lasting decrease in synaptic strength on the further ability of the neural circuit to process patterned information and encode it, we measured longterm potentiation (LTP). In these experiments, DL-TBOA treated slices potentiated to the same extent as non-treated slices after theta-burst stimulation, but showed a reduced threshold for plasticity when a milder stimulation was tested. The FSL rat model of depression, has previously been reported by our group to have decreased levels of glial glutamate transporters. Interestingly, these animals exhibit a similarly reduced threshold for LTP as observed at acute glutamate transporter blockade.
Taken together our results propose that an impairment in glutamate uptake triggers a dynamic neuronal response possibly orchestrated to constrain excitatory signaling; however, this adaptive process could drive the reorganization of key elements for plasticity within the synapse modifying its ability to process information.

Disclosures: E. Vazquez-Juarez: None. **M. Lindskog:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.02/A81

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Increased astrocytic membrane depolarization at reduced glutamate uptake is mediated by NMDA receptor activation

Authors: *I. SRIVASTAVA, M. LINDSKOG;
Karolinska Institutet, Stockholm, Sweden

Abstract: The large majority of synaptic activity in the brain consists of glutamate synaptic transmission. Astrocytes play an important role in regulating this synaptic transmission by taking up glutamate at the perisynaptic space through glutamate transporters (EAAT1 and EAAT2). Changes in EAAT levels have been found in cases of neurodegenerative diseases and depression thus affecting this balanced glutamatergic neurotransmission. Glutamate uptake through astrocytes is an electrogenic process and involves exchange of ions affecting the membrane potential of astrocytes. Moreover, astrocytes are known to respond to changes in extracellular potassium by membrane depolarization. However very few studies have explored the mechanisms affecting astrocyte membrane potential and its functional significance in the conditions of reduced glutamate uptake.
In patch-clamp recordings of astrocytes in hippocampal slices from rats we confirm that stimulation of Schaffer collaterals induce a long-lasting (> 200ms) depolarization of the astrocytic membrane that is completely blocked when blocking the K_{ir} with 100 µM $BaCl_2$. This is consistent with synaptic activity increasing extracellular K^+ levels and enhancing astrocytic K_{ir} currents. In the presence of 50µM DL-TBOA to block EAAT, the astrocytic depolarization is greatly enhanced. Surprisingly this effect is not due to increased levels of glutamate leading to increased synaptic activity, since we show that 50µM DL-TBOA reduces synaptic responses. Instead, we propose that increasing the levels of synaptic glutamate though blocking of EAAT reduces the AMPA-mediated synaptic response while NMDA receptor mediated currents increases, contributing to extracellular K^+ increase leading to enhanced astrocytic depolarization.

Disclosures: I. Srivastava: None. **M. Lindskog:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.03/A82

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: UCC RISE R25GM110513
ADA 1-19-IBS-300
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NIH-NIMHD-G12-MD007583
Title V PPOHA P031M105050

Title: Upregulation of astrocytic GLT-1, ischemic stroke and sex as a variable

Authors: *F. A. TEJEDA BAYRON[1], D. E. RIVERA-APONTE[1], C. J. MALPICA NIEVES[2], G. MALDONADO-MARTÍNEZ[3], Y. HERNANDEZ[1], S. N. SKATCHKOV[4], M. J. EATON[1]; [1]Biochem., [2]Physiol., [3]Retrovirus Res., [4]Biochemistry, Physiol., Univ. Central Del Caribe, Bayamón, Puerto Rico

Abstract: During ischemic stroke, glutamate and potassium are released, reuptake processes are impaired, and glutamate promotes excitotoxic neuronal death. Astrocytic GLT-1 is the major glutamate transporter responsible for removing excess glutamate from the extracellular space. A neuroprotective compound LDN/OSU 0212320 (LDN; a translational activator of GLT-1) has been developed with beneficial outcomes in epilepsy animal models. Similar to stroke, glutamate is released during epileptic seizures. The goal of the present study was to evaluate the effects of LDN on stroke-associated brain injury. Male and female mice (10-12 weeks) were subjected to unilateral focal ischemia induced in the sensorimotor cortex using the Rose Bengal photothrombotic method. Baseline sensorimotor performance was determined using the rung ladder walk behavioral test prior to the surgery and reevaluated 24 hours after the focal photothrombosis. Mice received an i.p. injection of either LDN (40 mg/kg) or vehicle, 24 hours before the focal photothrombotic surgery. Mice were decapitated and their brains removed 48 hours after receiving the focal lesion. The cerebellum was collected for Western blot analysis for GLT-1. Sections (1 mm thick) from the remaining brain were stained in a 5% solution of triphenyltetrazolium chloride (TTC), photographed and the size of the lesion was measured using Image J. After focal ischemia, we found that males treated with LDN displayed better sensorimotor performance in the right front and back left paws in comparison to those treated with vehicle only. In contrast, there was no difference between post-stroke performance on the rung ladder walk between LDN- or vehicle-treated female mice. The volume of the infarct for vehicle- and LDN- treated male mice was 44.4 ± 8.2 mm^3 (mean ± SEM; n=9) and 30.0 ± 5.1 mm^3 (n=9; $p<0.05$), respectively. In contrast, the infarct size was not significantly different

between vehicle- (40.5 ± 11.5 mm^3; n=9) and LDN-treated (33.5 ± 2.9 mm^3; n=9; n.s.) female mice. Surprisingly, LDN increased GLT-1 expression in young males, but not females. Taken together, our results indicate that the GLT-1 translational activator LDN improved outcomes after stroke in young adult male, but not in female mice.

Disclosures: F.A. Tejeda Bayron: None. **D.E. Rivera-Aponte:** None. **C.J. Malpica Nieves:** None. **G. Maldonado-Martínez:** None. **Y. Hernandez:** None. **S.N. Skatchkov:** None. **M.J. Eaton:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.04/A83

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Deutsche Forschungsgemeinschaft Research Fellowship (T.S.R.)
NIH grant AG006173 (D.J.S.)
NIH grant EY13079 (C.J.A.)
NIH grant NS066019 (P.A.R.)
NIH grant MH104318 (P.A.R.)

Title: Conditional inactivation of the glutamate transporter GLT-1 in neurons produces an age-dependent defect in synaptic transmission in the acute hippocampal slice

Authors: *T. S. RIMMELE[1], S. LI[2], D. J. SELKOE[3], C. J. AOKI[4,5], C. G. DULLA[6], P. A. ROSENBERG[1,7];
[1]F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA; [2]Neurol., Brigham and Women's Hosp., Boston, MA; [3]Neurol., Brigham & Women's Hosp, Boston, MA; [4]Ctr. Neural Sci., New York Univ., New York, NY; [5]Neurosci. Inst., NYU Langone Med. Ctr., New York, NY; [6]Neurosci., Tufts Univ. Sch. of Med., Boston, MA; [7]Program in Neurosci., Harvard Med. Sch., Boston, MA

Abstract: The tight regulation of extracellular glutamate by glutamate transporters is essential for the maintenance and fidelity of synaptic transmission as well as the survival of neurons. GLT-1 is the major glutamate transporter in the brain. Most GLT-1 is expressed in astrocytes, however, 5-10% of GLT-1 is expressed in neurons, primarily in excitatory presynaptic terminals. We generated a conditional neuronal GLT-1 knockout using synapsin 1-Cre (synGLT-1 KO) to elucidate the functions of GLT-1 expressed in neurons. We examined excitatory neurotransmission in acute hippocampal brain slices of synGLT-1 KO and wildtype (WT) littermates using electrophysiology combined with extracellular glutamate imaging, recording in the CA1 region. Stimulus-evoked field excitatory post-synaptic potentials (fEPSPs) from the

synGLT-1 KO were not significantly different from WT at 10-12 weeks of age. In contrast, at 18-20 weeks, fEPSPs were significantly smaller (53% reduction) in the synGLT-1 KO than in the WT (n=17-18; p=0.0083), and in some cases undetectable. We then studied paired pulse facilitation and found no significant difference in synGLT-1 KO at 18-20 weeks compared to WT (n=17-18; p= 0.4626). Next, we quantified stimulus-evoked changes in extracellular glutamate using a FRET based glutamate sensor. These glutamate imaging studies revealed an age-dependent reduction by 47% in evoked glutamate release in the synGLT-1 KO compared to WT (n=15-16; p=0.0260). These findings suggest that loss of GLT-1 expressed in presynaptic terminals leads to an age-dependent deficit in excitatory neurotransmission. We are currently carrying out patch clamp recordings to further pursue the cellular basis of the age-dependent deficit in synaptic transmission observed in the synGLT-1 KO. In a recent study focused on the cerebral cortex [McNair et al. (2019) *J Neurosci* doi: 10.1523/JNEUROSCI.0894-18.2019], we found in the synGLT-1 KO decreased aspartate content, decreased labeling of TCA cycle substrates by ^{13}C-glutamate, increased glycolysis, and redistributed synaptic and perisynaptic astrocytic mitochondria, suggesting that GLT-1 in axon terminals is required for utilization of glutamate by synaptic mitochondria. We hypothesize that age-dependent changes in synaptic mitochondrial metabolism might contribute to the impairment in excitatory synaptic transmission observed in the synGLT-1 KO.

Disclosures: **T.S. Rimmele:** None. **P.A. Rosenberg:** None. **C.G. Dulla:** None. **D.J. Selkoe:** None. **S. Li:** None. **C.J. Aoki:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.05/A84

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NSERC 298916-2011
CIHR PJT-159832

Title: Distinct roles of GLT-1 and EAAC1 in regulation of excitatory tonic current in MCH neurons

Authors: S. C. BOWES[1], C. BRIGGS[2,1], K. SEMBA[2], *M. HIRASAWA[1];
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Abstract: Melanin-concentrating hormone (MCH) neurons are known to promote sleep and weight gain. Glutamate provides an excitatory drive to promote the excitability of MCH neurons, which is regulated by glutamate transporters that maintain low levels of extracellular glutamate

and prevent overexcitation. Our recent study (Briggs et al. 2018 J. Neurosci.) showed that prolonged wakefulness leads to plasticity of glutamate transporter 1 (GLT-1) suggesting GLT-1's important role in sleep homeostasis. However, it remains unknown whether other glutamate transporters play a role and which types of glutamate receptors are regulated by specific glutamate transporters in MCH neurons. To answer these questions, whole cell patch clamp recording was performed on MCH neurons in acute rat brain slices. We identified a reversible tonic current (TC) induced by glutamate transporter blockade. TFB-TBOA (non-specific glutamate transporter inhibitor) displayed the largest TC (~700pA) superimposed with successive stepwise currents that occurred spontaneously or upon synaptic stimulation. DHK (GLT-1 inhibitor) induced a modest and smooth TC (~70pA). UCPH 101 (GLAST inhibitor) had no effect. These results indicate that GLT-1 (mainly astrocytic) and EAAC1 (neuronal) regulate glutamate signaling in MCH neurons, while GLAST (astrocytic) is not involved. Further, DHK-induced TCs were observed in the presence of TTX suggesting that rather than synaptically released glutamate, ambient glutamate underlies these currents, which is tightly regulated by GLT-1. In contrast, TTX significantly attenuated TBOA-induced TCs and abolished the stepwise currents, suggesting that these currents are likely due to synaptic activity that is regulated by EAAC1. Using specific receptor antagonists, we found that kainate receptors (KARs) mediate the majority of the DHK-induced TC. Conversely, the TBOA-induced TC was only partially blocked by the KAR antagonist, while largely inhibited by NMDAR and AMPAR antagonists. These findings strongly suggest that ambient glutamate preferentially activates KARs while synaptic glutamate activates AMPARs and NMDARs. In conclusion, our study shows a complex mechanism of excitatory transmission in MCH neurons mediated by distinct glutamate receptor pools whose activation is differentially regulated by glutamate transporter activity. Specifically, (i) ambient glutamate is responsible for TCs induced by glutamate transporter blockade, and (ii) GLT-1 and EAAC1 may regulate glutamate concentrations at different receptor pool locations. Given the known role of MCH neurons, this may have functional implications on sleep and energy homeostasis.

Disclosures: **S.C. Bowes:** None. **C. Briggs:** None. **K. Semba:** None. **M. Hirasawa:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.06/A85

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: U01AG054444
Bright Focus Foundation

Title: Investigating the mechanism underlying glutamate transporter EAAT2 localization to the plasma membrane triggered by pyridazine-derivatives

Authors: *Z. XU[1], X. WANG[2], J. B. FOSTER[1], K. HODGETTS[4], C.-L. G. LIN[3]; [1]Neurosci., The Ohio State Univ., Columbus, OH; [3]Dept Neurosci, [2]Ohio State Univ., Columbus, OH; [4]Neurol., Harvard Med. Sch., Cambridge, MA

Abstract: Glutamate transporter EAAT2 is primarily localized in peri-synaptic processes of astrocytes closely associated with excitatory synaptic contacts. EAAT2 plays a critical role in the homeostaticregulation of extracellular glutamate levels. EAAT2 also plays an essential role in cognitive memory functions. Increase ofEAAT2 expression and glutamate transport activityhas been considered a potential therapeutic strategy for many neurological diseases.We have discovered and developed a novel series of small molecules that can increase EAAT2 expression via a novel translational activation mechanism. These small molecules have been proved to be capable of normalizing glutamate dyshomeostasis and providing significant benefits in several disease models. However, the mechanism of compound action is still unclear. Our previous studies indicate that the compound target is located at the perisynaptic astrocytic processes. Binding of the compound to the target results in activation of local translation of EAAT2 and other mRNAs in the astrocytic processes. This leads to strengthening of the structural and functional plasticity of tripartite synapse. In the present study, we found that the compound also can trigger existing EAAT2 immediatelylocalized to the plasma membrane. This compound-mediated trafficking occurs before new EAAT2 are synthesized locally at astrocytic processes. Proteomic analysis of isolated cell surface proteins in the hippocampal regions revealed that a set of proteins were immediately localized to the plasma membrane following compound treatment. These proteins include (1) those involved in glutamate uptake, *e.g.*EAAT2, EAAT1, and Na^+/K^+ATPase; (2) those involved in membrane trafficking, *e.g.*synaptotagmin, syntaxin, and Rho guanine nucleotide exchange factors; (3) signaling molecules, *e.g.*G proteins; (4) cell adhesion molecules, *e.g.*neuronal cell adhesion molecules and neuroligins; and (5) receptors, *e.g.* glutamate ionotropic receptors and glutamate metabotropic receptors. Furthermore, this immediate membrane trafficking phenomenon was also observed in the mice that were placed in a Y-maze spatial recognition memory paradigm. These results indicate that immediate membrane trafficking represents a potential mechanism by which the tripartite synapse responds to changes in the synaptic microenvironment as well as modulates synaptic plasticity. We are currently investigating the underlying molecular mechanisms of compound-mediated trafficking. These results will be presented.

Disclosures: Z. Xu: None. **X. Wang:** None. **J.B. Foster:** None. **C.G. Lin:** None. **K. Hodgetts:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.07/A86

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: P30 CA010815

Title: Development of broad activity positive allosteric modulator of glial glutamate transporters EAAT1 and EAAT2

Authors: J. L. GREEN[1], A. KHATIWADA[1], P. A. N. REDDY[2], J. M. SALVINO[2], Y. FORSTER[3], L. BIGLER[3], W. F. SANTOS[4], *A. C. K. FONTANA[1];
[1]Pharmacol. and Physiol., Drexel Univ., Philadelphia, PA; [2]Wistar Inst., Philadelphia, PA; [3]Chem., Univ. of Zurich, Zurich, Switzerland; [4]Biol., Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: Excitatory amino acid transporters (EAATs) play a crucial role in the removal of synaptic glutamate to maintain extracellular concentrations below excitotoxic levels. Glutamate-mediated excitotoxicity has been associated with a number of CNS disorders, including stroke, traumatic brain injury, stroke, Amyotrophic Lateral Sclerosis, neuropathic pain, HIV-associated neurocognitive disorders (HAND) among others, as well as with addiction and mental health disorders. Glial transporters EAAT1 and EAAT2 are responsible for removal of the bulk of glutamate from the synapse, preventing over-excitation of post-synaptic neurons. Therefore, compounds that enhance its expression or function could serve as valuable neuroprotective agents. Previously, a compound isolated from the venom of the spider *Parawixia bistriata*, Parawixin10, was shown to have neuroprotective and anticonvulsant activities in *in vivo* epilepsy models of intrahippocampal N-methyl-D-aspartate microinjection, and pentylenetetrazole injection in the lateral ventricles. In this work, we report the identification of the chemical structure of this compound. In addition, we characterize its mechanism in glutamate transporter assays and report its neuroprotective properties in primary neuron-glia cultures. This compound is an acylpolyamine with a molecular weight of 589.44 Da. In transfected COS-7 cells, Parawixin10 increased activity of both glial transporters EAAT1 and EAAT2 in an allosteric manner. Parawixin10 was also shown to have neuroprotective properties in primary mixed neuron-glia cultures subjected to excitotoxic insults with glutamate and oxygen-glucose deprivation (OGD, an *in vitro* model of stroke). Future directions include molecular docking assays to determine the region of the transporters that Parawixin10 interacts with, and development of a medicinal chemistry campaign to improve its drug-like properties. This class of compounds can be developed into therapies for neurological diseases and conditions in which glutamate excitotoxicity is involved.

Disclosures: **J.L. Green:** None. **A. Khatiwada:** None. **P.A.N. Reddy:** None. **J.M. Salvino:** None. **Y. Forster:** None. **L. Bigler:** None. **W.F. Santos:** None. **A.C.K. Fontana:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.08/B1

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Intramural Research at the NIH, NIMH

Title: Amphetamine-induced internalization of the glutamate transporter, EAAT3, regulates the behavioral actions of amphetamines

Authors: *S. M. UNDERHILL[1], T. WIGSTROM[1], S. H. MILLAN[1], P. D. HULLIHEN[1], C. T. RICHIE[2], B. K. HARVEY[2], S. G. AMARA[1];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]NIDA, NIH, Baltimore, MD

Abstract: The actions of amphetamines (AMPHs) are due to modulations of several neurotransmitter systems including dopamine and glutamate. AMPH stimulates internalization of the plasma membrane dopamine transporter, DAT, which contributes to modulation of extracellular dopamine levels. We have found that AMPH also stimulates internalization of the neuronal plasma membrane glutamate transporter, EAAT3. AMPH-stimulated endocytosis of EAAT3 mediates increases in extracellular glutamate as well as enhances the activation of glutamate receptors on dopamine neurons. In order to investigate the behavioral effects of EAAT3 endocytosis, we examined AMPH-mediated hyperlocomotion in mouse and rat animal models.
Similar to previous observations, we found that global EAAT3 knockout mice had a diminished response to AMPH. Both male and female mice that lack EAAT3 expression did not hyperlocomote in response to 2 mg/kg AMPH. We did find, however, that the dopamine transporter, DAT, was still internalized in response to AMPH. Similarly, the upstream mediator of both DAT and EAAT3 endocytosis, the small GTPase RhoA, was still activated in AMPH-treated acute brain slices from these animals. To further refine our understanding of AMPH-induced EAAT3 trafficking, we identified a unique sequence on the C-terminus of EAAT3 that mediates AMPH-induced internalization. Exogenous introduction of this sequence fused to a TAT-peptide prevents AMPH-induced endocytosis of EAAT3, but has no effect on the DAT. We designed a Cre-dependent AAV vector that expresses this short peptide fused to GFP and stereotactically introduced it to the ventral tegmental area (VTA) of DAT-Cre rats. AMPH-treated rats that were expressing the exogenous protein had a decreased response to AMPH, 2 mg/kg. Adult (12-24 week old) female rats did not hyperlocomote at the same level as their sham-treated litter-mates. Male rats treated with the viral EAAT3 sequence exhibited less rearing

in response to AMPH compared to their littermate controls.
These data indicate that AMPH-mediated internalization of EAAT3 in dopamine neurons of the VTA is an important component of the behavioral outcomes of the psychostimulant treatment.

Disclosures: S.M. Underhill: None. **T. Wigstrom:** None. **S.H. Millan:** None. **P.D. Hullihen:** None. **C.T. Richie:** None. **B.K. Harvey:** None. **S.G. Amara:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.09/B2

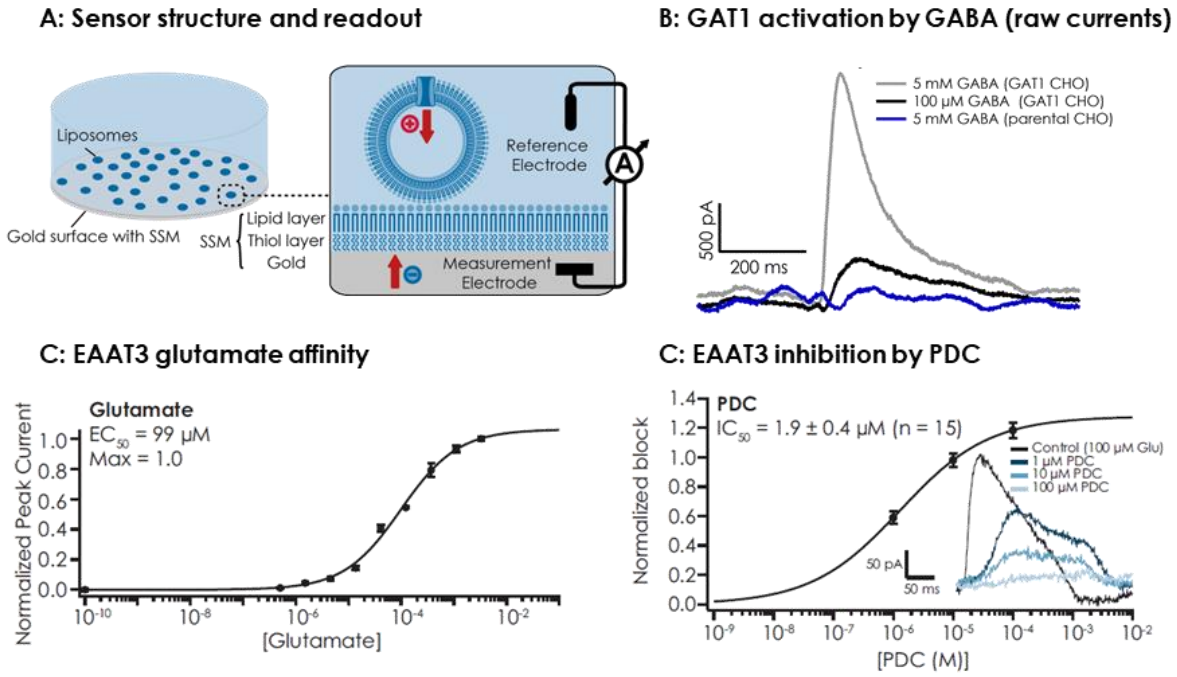
Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: GABA and glutamate re-uptake transporters GAT1 and EAAT3 functionally investigated using a high throughput system

Authors: M. BARTHMES[1], R. RIZZETTO[3], A. BAZZONE[1], A. ROSSIGNOLI[3], J.-F. ROLLAND[3], *J. L. COSTANTIN[2], N. FERTIG[1];
[1]Nanion Technologies, Munich, Germany; [2]Nanion Technologies, Newark, CA; [3]Axxam SpA, Milan, Italy

Abstract: GAT1 (SLC6A1) and EAAT3 (SLC1A1) are secondary active neurotransmitter transporters coupled to the ion gradients established by the NaK ATPase. Both are expressed in neurons and play a major role in the depletion of neurotransmitters GABA and glutamate from the synaptic cleft, thereby taking part in termination of synaptic transmission, maintaining the ambient extracellular neurotransmitter concentration and neurotransmitter recycling through reuptake. This renders these transporters interesting pharmacological targets. GAT1 is linked to epilepsy, schizophrenia, anxiety and ADHD. It is strongly inhibited by anti-epileptic drugs. Subtype specific inhibition of EAAT3 is assumed to be beneficial during phases of insufficient energy supply by preventing reversal glutamate transport. Therefore screening compounds to find inhibitors of these transporters is of high interest.
Here we established and evaluated new assay formats for functional investigation of GAT1 and EAAT3 activity, as well as identification and characterization of their inhibitors. We used purified plasma membrane vesicles from CHO cells expressing EAAT3 or GAT1, which where immobilized on a solid supported membrane-based sensor system. Activity was triggered by application of GABA and glutamate, respectively. The charging of the vesicles caused by the electrogenic transport was measured. To obtain sensors with 100% right side out oriented protein we established a whole-cell assay format using the GAT-CHO cells. The EAAT3 assay was transferred to a high throughput system in 96-well format. Using this system we determined the Km of glutamate to 99 µM and of GABA to 0.5 mM. We investigated six inhibitors of EAAT3 and report similar or lower IC_{50}s compared to literature, thus demonstrating the sensitivity of our

assay system (e.g. HIP-B 3.3 ± 1.4 µM, MPCD 7.2 ± 1.5 µM, PDC 1.9 ± 0.4 µM). Furthermore, we used similar procedures to establish reliable assays to investigate endogenous EAAT and GAT activity in iPSC derived neurons using whole cells.



Disclosures: **M. Barthmes:** A. Employment/Salary (full or part-time):; Nanion Technologies. **R. Rizzetto:** A. Employment/Salary (full or part-time):; Axxam SpA. **A. Bazzone:** A. Employment/Salary (full or part-time):; Nanion Technologies. **A. Rossignoli:** A. Employment/Salary (full or part-time):; Axxam SpA. **J. Rolland:** A. Employment/Salary (full or part-time):; Axxam SpA. **J.L. Costantin:** A. Employment/Salary (full or part-time):; Nanion Technologies. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.10/B3

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NINDS NS104478

Title: Genetically encoded voltage indicators reveal large, fast voltage fluctuations in cortical astrocyte processes that modulate excitatory neurotransmission

Authors: M. ARMBRUSTER[1], Y. ADAM[2], A. E. COHEN[3], ***C. G. DULLA**[4];
[1]Neurosci., Tufts Univ., Boston, MA; [3]Chem. and Chem. Biol., [2]Harvard Univ., Cambridge, MA;
[4]Tufts Univ. Sch. of Med., Boston, MA

Abstract: Astrocytes remove the excitatory neurotransmitter glutamate from the extracellular space following neuronal activity via sodium-driven, voltage-dependent excitatory amino acid transporters (EAATs). Robust glutamate uptake by EAATs ensures the temporal and spatial fidelity of glutamate signaling. *We recently found that neuronal activity rapidly (within milliseconds) and reversibly slows glutamate uptake in the adult cerebral cortex.* This slowing prolongs neuronal NMDA responses, consistent with prolonged extracellular glutamate dynamics, and is highly dependent on the frequency and duration of stimulation. We believe this may have important consequences for neurotransmission, extrasynaptic receptor activation, and synaptic plasticity. Based on this finding, we hypothesized that neuronal activity induces microdomain-level changes in astrocyte membrane potential (V_m) that locally modulate EAAT function. GLT1 is the predominant astrocytic EAAT in the adult forebrain, is abundantly expressed, and ensures that glutamate in the extracellular space is rapidly removed. Once bound to EAATs, the transport of glutamate into the astrocyte is both sodium-driven and voltage-dependent. Under normal conditions, astrocytes are hyperpolarized (-80 mV) due to their high permeability to potassium. However, neuronal activity increases extracellular potassium, $[K^+]_e$, and astrocyte V_m is especially sensitive to $[K^+]_e$ changes. Therefore, it is plausible that neuronal activity could alter EAAT function by depolarizing astrocytes. Changes in astrocytic V_m may be especially relevant in fine astrocytic processes, where EAATs are concentrated, and where larger, focal changes in V_m may occur. A major challenge to testing our hypothesis, however, is an inability to monitor astrocyte V_m at distal processes due to low membrane resistance and fine-scale process morphology. Overcoming this challenge is important because astrocyte distal processes are the site of synaptic interaction and EAATs localization. To detect distal changes in astrocyte V_m, we developed an approach to image V_m in astrocyte processes using genetically-encoded voltage indicator (GEVI) imaging. Utilizing astrocyte and neuron electrophysiological recording, optogenetic manipulation of astrocyte V_m, and astrocyte GEVI and calcium imaging we have generated preliminary data that supports our hypothesis that EAAT function can be modulated by activity-induced changes in astrocyte V_m. GEVI-based imaging suggests that voltage changes in astrocyte processes are 10 times larger in amplitude and return to baseline 10 times faster than changes recorded electrophysiologically at the soma.

Disclosures: C.G. Dulla: None. **M. Armbruster:** None. **Y. Adam:** None. **A.E. Cohen:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.11/B4

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Hussman Foundation grant HIAS#18004

Title: The correlation of parvalbumin expression, neuronal morphology, and synaptic activity

Authors: *R. F. NIESCIER, M. KILANDER, Y.-C. LIN;
Hussman Inst. For Autism, Baltimore, MD

Abstract: The disruption of excitatory and inhibitory (E/I) balance in the neuronal circuitry has been a key contributing factor in several neurological conditions, including autism spectrum disorders (ASD). Among all neurons, fast-spiking parvalbumin (PV) interneurons are one of the most vulnerable populations. Reduction in the expression of PV or the number of PV+ neurons has been observed in several animal models as well as in individuals with ASD. PV is a calcium chelating protein that buffers the excessive calcium during activity stimulation to maintain the proper neuronal response and function. Meanwhile, PV+ neurons exhibit complex axonal processes and form axosomatic synapses on the neighboring excitatory neurons to produce the maximal inhibition of the excitation. The reduction of PV expression may likely jeopardize the neuronal function by allowing extra calcium entry and thus compromising synaptic integrity. Therefore, we hypothesize that the expression of parvalbumin correlates with neuronal morphology and synaptic activity.
We used primary cultured neurons dissociated from the PV-tdTomato mice and examined the protein expression by immunostaining with the antibody against PV. We found a significant population of tdTomato+ neurons had low or non-detectable PV signals by antibody staining, although fluorescent In situ hybridization (FISH) confirmed PV expression. We further examined the morphology of tdTomato+ neurons by performing Sholl analysis and measured the spontaneous activity using genetically encoded calcium indicator (GECI). Additionally, after exposing neurons to L-glutamate we found that PV expression was rapidly up-regulated and coincided with an increase in ERK phosphorylation. We further engineered shRNA targeting PV to knock down PV proteins in cultured neurons. The neurite morphology and synaptic activity of neurons with PV knockdown were further analyzed . This study provides a potential explanation for why reduction in PV function may result in cellular defects observed in ASD.
Support: This study was supported by the Hussman Foundation grant HIAS#18004 to YCL.

Disclosures: R.F. Niescier: None. **M. Kilander:** None. **Y. Lin:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.12/B5

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: The effects of sevoflurane or desflurane general anesthesia on markers of neurotoxicity in the adult female monkey

Authors: *E. CUEVAS, S. M. BURKS, C. FOGLE, F. LIU, J. TALPOS;
NCTR-FDA, Jefferson, AR

Abstract: Sevoflurane (SEVO) and desflurane (DES) are commonly used gaseous anesthetics. Reports indicate that early life exposure to anesthesia with SEVO or DES, as well as other anesthetics, can cause neurodegeneration and lasting behavioral changes. It is thought that anesthetics influence the cerebral cortex, amygdala, and hippocampus to induce anesthesia, presumably by the activation of intra- and extracellular pathways that may also cause neurotoxicity. While general anesthesia is typically considered safe in healthy adults, this claim has never been thoroughly studied in the nonhuman primate. This evaluated the effects of SEVO and DES on CNS markers of neurodegeneration, neuroinflammation, oxidative stress, glutamatergic function / synapse formation, and autophagy. Peripheral changes in inflammation markers were also considered. Here, mature female rhesus monkeys were exposed to 9 h of SEVO (approximately 2.5% in medical grade air; n=5), or DES (approximately 5.7% in medical grade air, n=5), or a control condition (n=5). Four hours after exposure, the animals were sacrificed and brains collected. After dissection and tissue preparation, markers of neuronal (synaptophysin, NMDAR2A, caspase-3, DA, TH), glial (GFAP, IBA1), oxidative stress (RAGE, protein oxidation), and autophagy (LAMP-1, LAMP-2, LC3B) changes in the frontal cortex were evaluated using dot-blot and/or western-blot analysis. Inflammation markers (IL-6, IL-4, IL-1β, INF-γ, IL-8, MPC-1) in serum were also analyzed by ELISA. In general, SEVO and DES did not produce changes in the primary endpoints. However, SEVO produced a decrease in TH levels and DES decreased LAMP1 and LC3B in the frontal cortex. Both compounds decreased serum levels of IL-8. Unlike previous results in perinatal rhesus monkeys, these findings indicate that SEVO or DES exposure does not cause overt signs of neurotoxicity in the frontal cortex. However, decreased expression of LAMP1 and LC3B, which are involved in autophagy, after DES exposure does merit additional research. Similarly, a more detailed examination of the blood brain barrier and brain vasculature may be warranted based on the decreased levels of serum IL-8. (Funded by NCTR/FDA)

Disclosures: E. Cuevas: None. **S.M. Burks:** None. **C. Fogle:** None. **F. Liu:** None. **J. Talpos:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.13/B6

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH BP-ENDURE Research Fellowship

Title: Localization of glutamate-like immunoreativity in the nervous system of Biomphalaria glabrata: An intermediate host for schistosomiasis

Authors: *K. NIEVES[1,2], P. MÉNDEZ[1], A. OPPENHEIMER[1], M. W. MILLER[1,3];
[1]Inst. of Neurobio., San Juan, Puerto Rico; [2]Univ. of the Sacred Heart, San Juan, PR; [3]Dept. of Anat. & Neurobiology, Univ. of Puerto Rico, Med. Sci. Campus, San Juan, PR

Abstract: Approximately ten percent of the worldwide population lives at risk of contracting the parasitic disease, schistosomiasis, popularly known as “snail fever”. The digenetic trematode worm species *Schistosoma mansoni*, that is the causative of the most common form of intestinal schistosomiasis, requires the freshwater snail *Biomphalaria glabrata* to serve as its primary intermediate host. Within the snail, *S. mansoni* multiplies and develops into its cercarial stage which can infect humans. The infection of pulmonate snails by larval trematodes has been shown to alter host behavior. For this reason, a commercial antiserum against glutaraldehyde-BSA conjugate glutamate, was used (mouse monoclonal) to localize glutamate-like immunoreactivity in the central and peripheral nervous systems of *B. glabrata*. Glutamate-like immunoreactivity (GLUi) was observed throughout the central nervous system (CNS), with the greatest numbers in the buccal, left pedal as well as, left and right parietal ganglia. GLUi fibers were present in all nerves, connectives and commissures. These results suggest that the glutamate serves as a neurotransmitter in *B. glabrata.* Future experiments will explore whether glutamatergic signaling contributes to sensory responses to miracidium penetration or to behaviors that are altered following infection.

Disclosures: K. Nieves: None. **P. Méndez:** None. **A. Oppenheimer:** None. **M.W. Miller:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.14/B7

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Propagation of synaptic temperature fluctuations by glutamateelectro-diffusion

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Abstract: Brain temperature is strongly regulated by cerebral blood flow and shows fluctuations in response to stimuli and neuroactive drugs. Cells in the nervous system not only detect

environmental temperature changes through their unique temperature-sensitive molecular machineries but also muster an appropriate response to the temperature change to maintain their inherent functions. However, the mechanisms by which neurons produce, use and transfer heat are largely unknown. The focus of this study is the latter, namely how temperature gradients are transferred within the synaptic cleft, a process that can affect synaptic transmission by ultimately altering conductivity of post-synaptic ion channels. The dissolution of (charged or polar) neurotransmitters such as glutamate following release from presynaptic terminals within the extracellular fluid has been considered to be driven largely by diffusion. Furthermore, the electric fields of narrow synaptic clefts may also influence synaptic currents. However, how these processes causally relate to heat propagation remains poorly understood, mainly because events inside the cleft are beyond the powers of direct experimental observation. We use a non-equilibrium thermodynamical model comprised by a system of partial differential equations that describes the changes in intracleft temperature as a function of electrodiffusion of neurotransmitters. Numerical simulations suggest that transmitter release and propagation correspond to measurable thermal fluctuations ranging from tens to hundreds of mK within the cleft. The findings provide a plausible description for temperature changes during normal brain activity that are independent from those induced by blood circulation and provide correction-factors for temperature changes associated with diseases such as epilepsy and Parkinson's.

Disclosures: M. Soltanpour: None. **H. Noori:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.15/B8

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Lactate enhances NMDA receptor responses via two distinct mechanisms

Authors: F. LEMTIRI-CHLIEH[1], G. HERRERA-LÓPEZ[2], L. MOTTIER[3], H. MAHMOOD[3], ***H. FIUMELLI**[3], P. J. MAGISTRETTI[3];
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Abstract: Lactate is not only known as an energy substrate for neurons but it is also emerging as an important trophic signaling molecule regulating higher brain functions such as learning and memory and depression. Recent findings have shown that lactate potentiates NMDA receptor signaling and regulates gene expression related to synaptic plasticity.
Using patch clamp recordings in primary cultures of cortical neurons, we found that lactate rapidly enhanced both the amplitude and the inactivation time-constant (τ) of NMDA receptor currents (I_{NMDA}) evoked by brief applications of glutamate and glycine. The effect on the

amplitude was prevented by pre-treating neurons with a MCTs blocker or by including CaMKII inhibitors in the patch pipette, whereas the effect on τ was not.
Given the observation that CaMKII was required for the effect of lactate on the amplitude of I_{NMDA}, we recorded from two types of heterologous cells expressing functional NMDARs: CaMKIIα-expressing HEK293 cells and control HEK293 cells. The effect of lactate on the amplitude of I_{NMDA} was only seen in CaMKIIα-expressing HEK293 cells. This enhancement was dependent on MCT transport since its blockade prevented this effect. In contrast, the effect on the inactivation kinetics of NMDAR responses was always seen regardless of CaMKIIα expression.
To further investigate the intracellular mechanisms involved in lactate enhancement of I_{NMDA} amplitude, we interfered with the intracellular conversion of lactate into pyruvate using pharmacological LDH inhibitors and with CaMKIIα binding to NMDA receptors by expressing mutant NMDA receptor subunits. The results showed that blocking LDH or expressing GluN2B mutants prevented the increase in I_{NMDA} amplitude induced by lactate. Interestingly, loading HEK293 cells with NADH also occluded the effect of lactate on I_{NMDA} amplitude. In contrast, none of these treatments affected the change observed in τ.
Taken together, these results indicate that lactate generates two distinct effects on NMDAR responses. 1) An enhancement of the I_{NMDA} amplitude that requires intracellular uptake of lactate, an increase in the internal NADH/NAD+ ratio through LDH activity, and interaction with CaMKIIα. 2) A change in the inactivation kinetics that is
independent of lactate import possibly by directly acting on a yet unidentified extracellular target.

Disclosures: F. Lemtiri-Chlieh: None. **G. Herrera-López:** None. **L. Mottier:** None. **H. Mahmood:** None. **H. Fiumelli:** None. **P.J. Magistretti:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.16/B9

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Changes in synaptic NMDAR evoked currents reveal modifications in D-serine levels after inhibition of ASC-1 transporter

Authors: *P.-Y. SHIH, F. SEIBT, H. LAVREYSEN, J. D. PITA ALMENAR;
Janssen Pharmaceutica R&D Neurosci., Beerse, Belgium

Abstract: D-serine is a co-agonist of NMDA receptors (NMDARs) whose synaptic availability is potentially regulated by the alanine-serine-cysteine transporter-1 (ASC-1), a transporter having high affinity for D-serine and glycine. ASC-1 can operate bidirectionally, though the preferred

direction of ASC-1 under physiological condition remains uncertain. Here we used selective ASC-1 blockers to explore its modulatory effect on NMDAR currents (NMDAR-EPSCs) with whole-cell patch-clamp recordings in rat acute hippocampal and cortical slices. We firstly showed that the level of occupancy of the NMDAR glycine-binding site is near saturated by endogenous D-serine in the hippocampus. To enable full-range estimation of ASC-1 function, we used either miniature EPSC measurements or co-application of 7-chlorokynurenate (7-CK, a competitive glycine binding site antagonist) to lower the glycine-binding site occupancy level. Under both conditions, we clearly observed an enhancement of NMDAR-EPSCs with exogenous D-serine, but an attenuation when an ASC-1 blocker was applied. We also demonstrated that in acute slices of medial prefrontal cortex, where the NMDAR glycine binding site is unsaturated, ASC-1 blocker could not augment the NMDAR-EPSCs neither. Furthermore, although we confirmed the D-serine deficiency and its consequent occurrence of synaptic plasticity decline in animal models of aging, we could not restore this deficit with our ASC-1 blocker. Taken together, our findings provide evidence that D-serine efflux is the preferred direction of ASC-1 under multiple physiological conditions, which is required for optimal NMDAR function and synaptic activity.

Disclosures: **P. Shih:** None. **F. Seibt:** None. **H. Lavreysen:** None. **J.D. Pita Almenar:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.17/B10

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: AFOSR Grant 13RH14COR
AFOSR Grant 16RHCOR362

Title: Non-invasive brain stimulation affects rat hippocampal protein expression in an intensity and dose-dependent manner

Authors: *C. N. HATCHER-SOLIS[1], S. H. JUNG[1], R. MOORE[1], N. BECHMANN[1], S. HARSHMAN[1], J. MARTIN[1], R. JANKORD[2];
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Abstract: Transcranial direct current stimulation (tDCS) is the application of low intensity current through non-invasive electrodes placed on the head that target specific areas of the brain. Prevailing evidence indicates tDCS affects learning and memory in a polarity and intensity-dependent manner, but the regulatory mechanisms remain unclear. In this study, we examined the impact of stimulation polarity (anodal vs. cathodal) and intensity (250 uA vs. 500 uA) on protein expression in the rat hippocampal synapse. We hypothesized anodal tDCS would

significantly upregulate hippocampal proteins associated with learning and memory in an intensity-dependent manner and that cathodal stimulation would have opposing effects. To test this hypothesis, tDCS was applied to twenty-five adult, male Sprague Dawley rats randomly assigned to five treatment groups receiving sham, anodal 250 µA, anodal 500 µA, cathodal 250 µA, or cathodal 500 µA stimulation (n=5/group). Animals were euthanized two hours post-stimulation and hippocampal tissue was collected. Synaptoneurosomes were biochemically purified from hippocampal tissue and individual protein abundances were quantified using bottom up liquid chromatography mass spectrometry analysis by researchers blinded to the treatment groups. Multiple bioinformatics methods were utilized to evaluate the protein expression differences between the treatment groups. Proteomic analysis identified 3069 differentially expressed proteins, of which 378 were differentially regulated by tDCS. DAVID Bioinformatics Database identified that compared to sham stimulation, anodal tDCS significantly upregulated clusters associated with the postsynaptic density, synapse, and dendrite. Anodal 500 µA tDCS further upregulated clusters associated with glutamatergic signaling and synaptic plasticity in the hippocampus. However, cathodal stimulation upregulated pathways associated with gamma-aminobutyric acid (GABA) signaling and the immune response. Ingenuity pathway analysis further showed anodal tDCS significantly enhanced pathways associated with calcium and glutamate receptor signaling, while cathodal stimulation upregulated pathways associated with GABA receptor signaling. Network analysis is ongoing. Our data provide evidence that tDCS modifies hippocampal protein expression in a polarity and intensity-dependent manner that may promote learning and memory. This work has identified multiple candidate protein targets and regulatory pathways for the effects of tDCS on learning and memory.

Disclosures: C.N. Hatcher-Solis: None. **S.H. Jung:** None. **R. Moore:** None. **N. Bechmann:** None. **S. Harshman:** None. **J. Martin:** None. **R. Jankord:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.18/B11

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Queen Elisabeth medical foundation
Willy Gepts foundation
Vrije Universiteit Brussel- SRP

Title: System x_c^--deficiency prevents age-related hippocampal dysfunction in mice

Authors: *L. VERBRUGGEN[1], E. BENTEA[1], A. VILLERS[2], O. LARA[1], G. ATES[3], D. DE BUNDEL[1], H. SATO[4], L. RIS[2], A. MASSIE[1];

[1]Vrije Univ. Brussel, Brussel, Belgium; [2]Univ. of Mons, Mons, Belgium; [3]Salk Inst. For Biol. Studies, San Diego, CA; [4]Niigata Univ., Niigata, Japan

Abstract: System x_c^-, with xCT as specific subunit, is an astrocytic antiporter that imports cystine in exchange for glutamate. System x_c^- is enhanced following oxidative stress and inflammation, both present in the aged brain. While we could not detect changes in xCT protein expression in the hippocampus of aged (20-24-month old) compared to adult (3-4-month old) C57BL/6 mice, xCT mRNA was significantly increased in hippocampus of 13-month old compared to 9-month old SAMP8 mice (model for accelerated aging). As system x_c^- releases glutamate into the extrasynaptic space, changes in its function can modulate glutamatergic neurotransmission. Moreover, enhancement of system x_c^- might induce neurological dysfunction, as it could decrease the threshold for glutamate toxicity (by releasing glutamate) and modulate neuroinflammation (by driving the pro-inflammatory microglial phenotype). We therefore investigated the role of system x_c^- in hippocampal function and how it can affect age-related hippocampal impairment. In the Barnes maze set-up, a behavioral test used for evaluating hippocampal function, adult xCT+/+ and xCT-/- mice show identical spatial learning and memory capacities. However, contrary to aged $xCT^{+/+}$ mice, the majority of aged $xCT^{-/-}$ mice learn to use the hippocampus-dependent direct search strategy in the Barnes maze set-up and they preserve this memory till 5 days after the last training session, comparable to adult mice. In the novel object location recognition task, loss of system x_c^- induces impairment of spatial memory in adult mice. However, whereas aging negatively affects performance of $xCT^{+/+}$ mice in this task, aged $xCT^{-/-}$ mice perform better compared to adult $xCT^{-/-}$ mice. In line with these behavioral data, basal hippocampal neurotransmission is reduced in adult $xCT^{-/-}$ mice, while the age-related decrease in basal synaptic transmission as well as the age-induced changes in LTP that are observed in $xCT^{+/+}$ mice, are prevented in the absence of system x_c^-. To conclude, our results show that the hippocampal aging process is fundamentally different in mice lacking system x_c^-. While system x_c^- seems to be important for certain aspects of hippocampal function in adult animals, it can become harmful during the aging process and contribute to age-related memory decline.

Disclosures: L. Verbruggen: None. **E. Bentea:** A. Employment/Salary (full or part-time):; UCB pharma. **A. Villers:** None. **O. Lara:** None. **G. Ates:** None. **D. De Bundel:** None. **H. Sato:** None. **L. Ris:** None. **A. Massie:** None.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.19/B12

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Queen Elisabeth Medical foundation
Willy Gepts Foundation
Vrije Universiteit Brussel - SRP
Fund Scientific Research Flanders - FWO

Title: System x_c^- - deficiency extends life-span and modulates aging of the immune system in mice

Authors: *A. MASSIE[1], L. VERBRUGGEN[1], O. LARA[1], J. DE MUNCK[1], P. JANSSEN[1], L. DE PAUW[1], H. SATO[2], G. ATES[3], R. NJEMINI[1], S. KOBAYASHI[4], J. L. AERTS[1], E. BENTEA[1];
[1]Vrije Univ. Brussel, 1090 Brussels, Belgium; [2]Niigata Univ., Niigata, Japan; [3]Salk Inst. For Biol. Studies, San Diego, CA; [4]Yamagata Univ., Yamagat, Japan

Abstract: The cystine/glutamate antiporter, with xCT as specific subunit, is mainly expressed in the central nervous system and tissues related to the immune system. Although system x_c^- has been proposed as therapeutic target for the treatment of cancer as well as several (age-related) neurological disorders, the function of system x_c^- in 'healthy' aging has never been studied. We confirmed the oxidative shift that was reported by Sato et al. (2005) to occur in the plasma of mice with a genetic deletion of xCT ($xCT^{-/-}$mice) and this seemed to be even more pronounced in aged $xCT^{-/-}$mice, compared to age-matched $xCT^{+/+}$littermates. Cystine plasma levels were not only increased in young $xCT^{-/-}$mice compared to $xCT^{+/+}$ littermates, but also significantly increased with aging in $xCT^{-/-}$ mice, contrary to $xCT^{+/+}$ mice. Moreover, cysteine levels significantly dropped with aging in $xCT^{-/-}$ mice, inducing a shift towards a more oxidative state. However, despite this oxidative shift that suggests accelerated aging, we observed a significantly increased median life-span in $xCT^{-/-}$ mice, compared to $xCT^{+/+}$ mice. The peripheral immune system and the central nervous system communicate in a bidirectional way. The functioning of both systems is affected by aging and can be modulated by system x_c^- We therefore investigated the effect of system x_c^- - deficiency on age-related hippocampal impairment (Verbruggen et al., sfn 2019 poster) as well as on exacerbation of the inflammatory response and immune senescence. Whereas aging induces an overall increase in the systemic response to a low-dose LPS injection (0.2mg/kg, i.p.), this effect was attenuated by the genetic deletion of xCT: aged $xCT^{-/-}$ mice have a smaller maximum drop in body temperature as seen at 4h and 6h after LPS administration and show reduced levels of peripheral pro-inflammatory cytokines at 3h post-LPS injection. Whether this can be translated to reduced neuroinflammation is currently being investigated. Aging also results in a disturbance of relative proportions of cells of both the innate and the adaptive immune system. Preliminary data show that the absence of system x_c^- during the aging process affects some of these age-related changes.To conclude, the positive effects of absence of system x_c^- during the aging process - both on life-span and hippocampal function - might in part be mediated by a reduced age-related systemic pro-inflammatory environment.

Disclosures: A. Massie: None. **L. Verbruggen:** None. **O. Lara:** None. **J. De Munck:** None. **P. Janssen:** None. **L. De Pauw:** None. **H. Sato:** None. **G. Ates:** None. **R. Njemini:** None. **S. Kobayashi:** None. **J.L. Aerts:** None. **E. Bentea:** A. Employment/Salary (full or part-time):; UCB-Pharma.

Poster

032. Glutamate Transport and Signaling

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 032.20/B13

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: German Research Foundation (DFG) SPP-1655
Internal funding from Max Planck Society

Title: Link extracellular glutamate signaling to the neuro-glio-vascular dynamic interaction with multi-modal fMRI

Authors: *Y. JIANG, X. CHEN, P. PAIS, X. YU;
High Field Magnetic Resonance, Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany

Abstract: Here, we expressed genetically encoded fluorescent reporter iGluSnFR for extracellular glutamate (Glu) sensing and calcium indicator GCaMP6f for calcium sensing. We first acquired evoked neuronal calcium and Glu signals with simultaneous fMRI from the FP-S1 of two hemispheres, respectively. **Fig. 1A** shows the fMRI maps and the time course of BOLD signal, which is simultaneous acquired with the neuronal calcium and Glu spikes. Evoked neuronal spikes showed iGluSnFR with a more rapid temporal feature than neuronal GCaMP (**Fig. 1 B**). Also, the amplitude of the evoked Glu spike increased proportionally to the BOLD signals as a function of the stimulation intensity (**Fig. 1 C**).
Besides the neuronal calcium, the evoked astrocytic calcium and Glu spikes were acquired with fMRI simultaneously (**Fig. 2 A**). Interestingly, we also observed the baseline drift of the Glu during the stimulation. This Glu baseline drift signal is increased proportionally upon the stimulation duration (**Fig. 2B**) and frequency (**Fig. 2C**). The linkage between the Glu with neuronal/astrocytic calcium and BOLD may indicate the clearance of Glu following synaptic Glu release or potential hemodynamic responses. Future study will further clarify the source for the Glu baseline drop to decipher the neurovascular coupling events.
This platform offers us a more thorough interpretation of source signal contribution to fMRI; thus, would expand our understanding of the neurovascular coupling through the neuro-glio-vascular network in the animal brain.

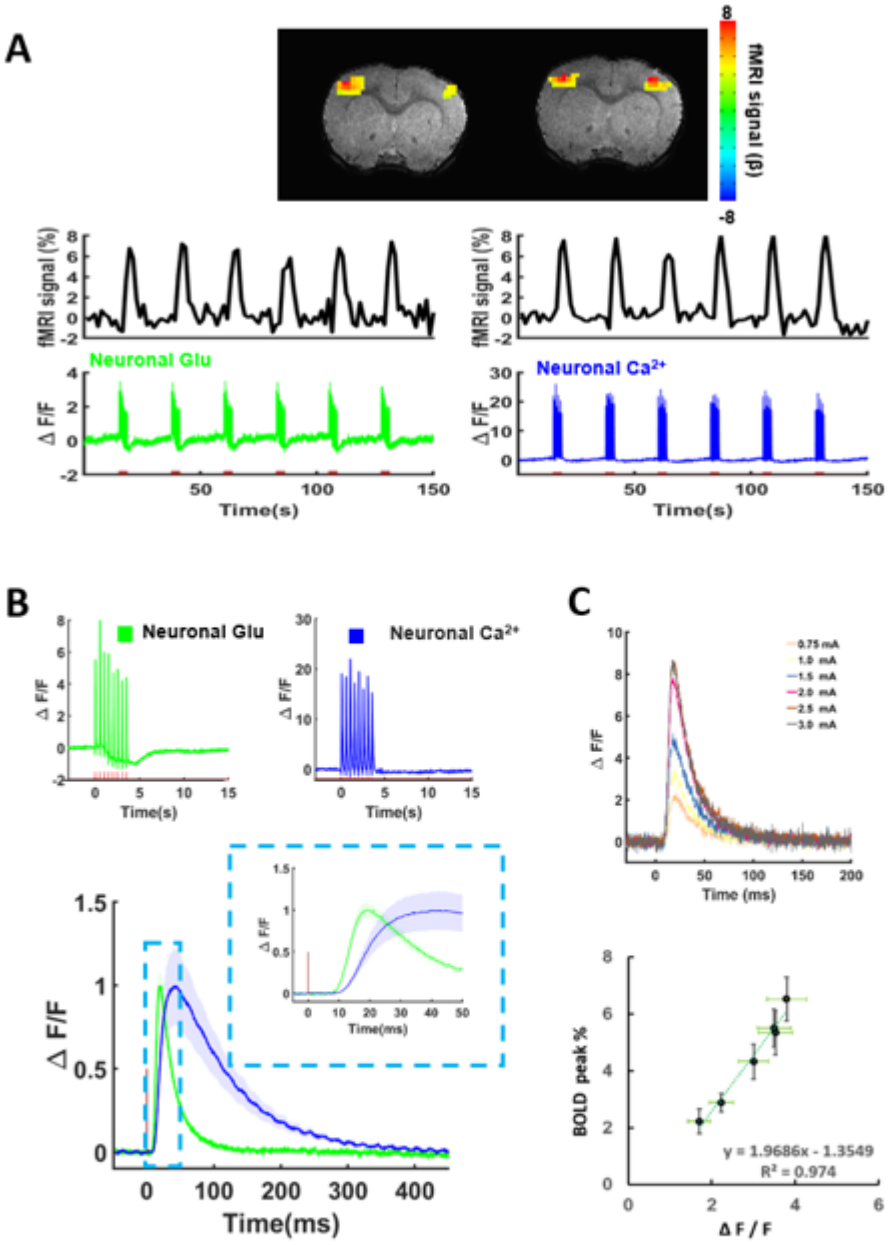

Figure 1. Characterizations of iGluSnFR and neuronal GCaMP responses and BOLD signal in rat somatosensory cortex by evoked forepaw electrical stimulation. (A) The BOLD fMRI signal and representative time course of neuronal Glu and calcium. Temporal features of sensory response of the evoked signal by multiply electrical forepaw stimuli (upper panel) in one trial and one pulse (lower panel, n=6). (C) The averaged The averaged Glu and BOLD peak signal dependence upon different amplitude (n=9).

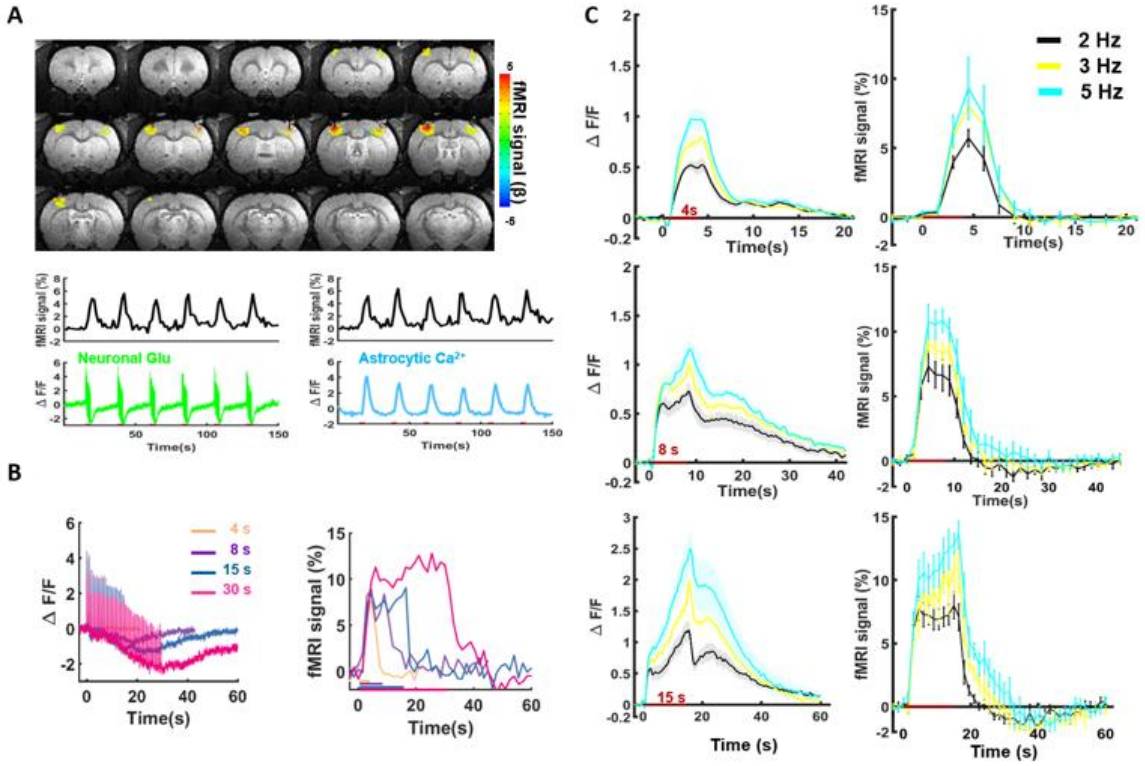

Figure 2. Comparison of glutamate, astrocytic calcium, and BOLD responses. (A) The whole brain fMRI map and time course of iGluSnFR and astrocytic expressed in rat somatosensory cortex. (B) The representative stimulation duration-dependent Glu baseline drift and BOLD signal (C) The comparison of Glu drift shape and BOLD signal at different frequency (2,3,5 Hz with duration of 4, 8, 15s, n=7).

Disclosures: **Y. Jiang:** None. **X. Chen:** None. **P. Pais:** None. **X. Yu:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.01/B14

Topic: B.03. G-Protein Coupled Receptors

Title: Stimulation of polyphosphoinositide hydrolysis by the oxytocin receptor agonist, carbetocin, in brain tissue

Authors: V. M. BUSÀ[1], L. DI MENNA[3], J. MAIRESSE[4,5,6], S. NOTARTOMASO[3], M. ZINNI[4], A. TRAFICANTE[3], G. BATTAGLIA[1,3], O. BAUD[4,5,6], S. MACCARI[2,7], B. CHINI[8],

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Abstract: Oxytocin receptors are involved in the regulation of developmental processes in the CNS, including the expression of the potassium/chloride cotransporter, KCC2, in neurons (Leonzino et al., Cell Rep., 2016). This is promising for the treatment of CNS disorders, including autism, in which the developmental shift in $GABA_A$ receptor function is impaired (Ben-Ari, Front. Cell. Neurosci., 2018). In addition, activation of oxytocin receptors in the CNS regulates behavioral and endocrine responses to stress, corrects the phenotype induced by perinatal stress in rodents (Gatta et al., Neurotoxicol., 2018), and reduces perinatal brain damage (Mairesseet al., Glia, 2019). Some of the features of oxytocin receptors are shared by mGlu5 metabotropic glutamate receptors, which are highly expressed and functional during early postnatal life and regulate KCC2 expression during development and in the adult life (Notartomaso et al., Neuropharmacol., 2017). mGlu5 receptors are coupled to $G_{q/11}$ proteins, and their activation leads to polyphosphoinositide (PI) hydrolysis with ensuing formation of inositol-1,4,5-trisphosphate and diacylglycerol. mGlu5-receptor mediated PI hydrolysis in brain tissue is robust early after birth and declines with age (Nicoletti et al., PNAS USA, 1986). Oxytocin receptors are coupled to either $G_{q/11}$ or G_i proteins in a context- and agonist-dependent fashion (Jurek and Neumann, Physiol. Rev., 2018), but there is no direct evidence that oxytocin receptors stimulate PI hydrolysis and interact with mGlu5 receptors. We report here that the oxytocin receptor agonist, carbetocin (10 nM or 1 μM), was able to enhance inositol phosphate formation in both cortical and hippocampal slices of mice at postnatal day 9. Using cortical slices, we studied the functional interaction between oxytocin and mGlu5 receptors using carbetocin and DHPG as respective orthosteric agonists. DHPG stimulated PI hydrolysis to a much greater extent than carbetocin (up to 7-8 fold *vs.* 40%). However, DHPG showed a greater efficacy in stimulating PI hydrolysis when combined with carbetocin in cortical slices. Amplification of DHPG-stimulated PI hydrolysis was seen with maximally effective concentrations of DHPG (100 μM) and not with lower concentrations, suggesting that activation of oxytocin receptors enhances mGlu5 receptor coupling to PI hydrolysis rather than the affinity of mGlu5 receptors for DHPG. These data provide the first evidence that oxytocin receptors are coupled to PI hydrolysis in brain tissue and may interact with mGlu5 receptors. We are currently examining the nature of this interaction and its functional significance in developmental processes.

Disclosures: V.M. Busà: None. **L. Di Menna:** None. **J. Mairesse:** None. **S. Notartomaso:** None. **M. Zinni:** None. **A. Traficante:** None. **G. Battaglia:** None. **O. Baud:** None. **S. Maccari:** None. **B. Chini:** None. **F. Nicoletti:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.02/B15

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH Grant MH118258

Title: Signaling and ionic mechanisms by which activation of oxytocin receptors increases neuronal excitability in the central amygdala

Authors: *S. LEI, B. HU, C. A. BOYLE;
Biomed. Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: Oxytocin (OXT) is a nonapeptide that exerts anxiolytic effects in the brain. The amygdala is an important structure involved in the modulation of fear and anxiety. A high density of OXT receptors (OXTRs) has been detected in the capsular (CeC) and lateral (CeL) nucleus of the central amygdala (CeA). Previous study has demonstrated that activation of OXTRs induces remarkable increases in neuronal excitability in the CeL/C. However, the signaling and ionic mechanisms underlying OXTRs-induced facilitation of neuronal excitability have not been determined. We found that activation of OXTRs in the CeL/C increased action potential firing frequency recorded from neurons in these regions via inhibition of the inwardly rectifying K^+ (Kir) channels. The functions of phospholipase Cβ (PLCβ) and protein kinase C (PKC) were necessary, whereas neither extracellular Ca^{2+} influx nor intracellular Ca^{2+} release was required for OXTRs-induced augmentation of neuronal excitability. Activation of OXTRs also inhibited Kir channels recorded from CeL/C neurons and application of inhibitors for PLCβ and PKC blocked OXTR-induced inhibition of Kir channels. The axons of CeL make GABAergic synapses onto the neurons in the medial nucleus (CeM) of the CeA and activation of OXTRs in the CeL enhanced the frequency of sIPSCs recorded from the neurons in the CeM. We also found that the functions of the Kir channels, PLCβ and PKC were required for OXTRs-induced augmentation of sIPSCs. Our results provided a novel signaling and ionic mechanism to explain OXT-mediated neural network activity in the amygdala and possibly its anxiolytic effects.

Disclosures: S. Lei: None. **B. Hu:** None. **C.A. Boyle:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.03/B16

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: KAKENHI 19K07287

Title: Excitatory effect of bradykinin on intrinsic neurons of the rat heart

Authors: S. ARICHI[1], S. SASAKI-HAMADA[2], Y. KADOYA[3], M. OGATA[2], *H. ISHIBASHI[2,1];
[1]Kitasato Univ. Grad. Sch. of Med. Sci., Sagamihara-City, Japan; [2]Dept. of Physiol., Kitasato Univ, Sch. Allied Hlth. Sci., Sagamihara-City, Japan; [3]Dept. of Anatom. Sci., Kitasato Univ., Sagamihara-City, Japan

Abstract: The heart is innervated by autonomic nervous system, and an extensive neural network exists in the heart. Although bradykinin (BK) has negative inotropic and chronotropic properties of cardiac contraction, the direct effect of BK on the intrinsic neural network of the heart is still unclear. In the present study, therefore, the effect of BK on the intracardiac ganglion neurons acutely isolated from rats was investigated using perforated patch-clamp recording technique.
Under current-clamp conditions, application of 0.1 μM BK depolarized the membrane, accompanied by repetitive firing of action potentials. This BK action was fully inhibited by the B_2 receptor antagonist Hoe-140, but not by the B_1 receptor antagonist des-Arg^9-[Leu^8]-BK. The BK response was mimicked by the B_2 receptor agonist [Hyp^3]-BK. In addition, the analysis of immunofluorescence revealed all of the isolated neurons to be positive for B_2 receptor, while only 15.3 % neurons were positive for B_1 receptors.The BK-induced depolarization was inhibited by the phospholipase C inhibitor U-73122. BK evoked inward currents under voltage-clamp conditions at a holding potential of -60 mV. Removal of extracellular Ca^{2+} markedly increased the BK-induced currents, suggesting an involvement of Ca^{2+}-permeable non-selective cation channels. The activation of muscarinic receptors by oxotremorine-M (OxoM) also elicited extracellular Ca^{2+}-sensitive cationic currents. The peak amplitude of inward current evoked by 0.1 μM BK was comparable to that induced by 1 μM OxoM. Co-application of 0.1 μM BK and 1 μM OxoM elicited the inward current whose peak amplitude was almost the same as the response elicited by OxoM alone. BK also reduced the amplitude of M-current deactivation induced by a hyperpolarizing step from a holding potential of -20 mV to -60 mV, while the effect of M-current inhibitor XE-991 was very similar to the BK action on the M-current, affecting neither resting membrane potential nor the BK-induced depolarization. From these results, we

suggest that BK regulates excitability of intrinsic cardiac neurons by both an activation of non-selective cation channels and an inhibition of M-type K^+ channels through B_2 receptors.

Disclosures: S. Arichi: None. **S. Sasaki-Hamada:** None. **Y. Kadoya:** None. **M. Ogata:** None. **H. Ishibashi:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.04/B17

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: A novel cell-penetrating peptide protects against neuron apoptosis after cerebral ischemia by inhibiting the nuclear translocation of annexin A1

Authors: *M. MENG;
Dept. of Neurobio., Tongji Med. Col., Wuhan, China

Abstract: Nuclear translocation of annexin A1 (ANXA1) has recently been reported to participate in neuronal apoptosis after cerebralischemia. Prevention of the nuclear translocation of ANXA1 should therefore inhibit neuronal apoptosis and protect againstcerebral stroke. Here, we found that, in the repeat III domain of ANXA1, the amino-acid residues from R228 to F237function as a unique nuclear translocation signal (NTS) and are required for nuclear translocation of ANXA1. Intriguingly,we synthesized a cell-penetrating peptide derived by conjugating the trans-activator of transcription (Tat) domain to the NTSsequence. This Tat-NTS peptide specifically blocked the interaction of ANXA1 with importin β and, consequently, thenuclear translocation of ANXA1 without affecting the nucleocytoplasmic shuttling of other proteins. The Tat-NTS peptideinhibited the transcriptional activity of p53, decreased Bid expression, suppressed activation of the caspase-3 apoptosispathway and improved the survival of hippocampal neurons subjected to oxygen-glucose deprivation and reperfusionin vitro. Moreover, using a focal brain ischemia animal model, we showed that the Tat-NTS peptide could be efficientlyinfused into the ischemic hippocampus and cortex by unilateral intracerebroventricular injection. Injection of the Tat-NTSpeptide alleviated neuronal apoptosis in the ischemic zone. Importantly, further work revealed that administration of the Tat-NTS peptide resulted in a dramatic reduction in infarct volume and that this was correlated with a parallel improvement inneurological function after reperfusion. Interestingly, the effects of Tat-NTS were injury specific, with little impact onneuronal apoptosis or cognitive function in sham-treated nonischemic animals. In conclusion, based on its profoundneuroprotective and cognitive-preserving effects, it is suggested that the Tat-NTS peptide represents a novel and potentiallypromising new therapeutic candidate for the treatment of ischemic stroke.

Disclosures: M. Meng: None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.05/B18

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH Grant 5R56MH114313-02
NIH Grant 1R21DA045825-01A1

Title: A comparison of unbiased and biased neuropeptide S receptor agonists in rats

Authors: *A. M. WOJCIECHOWSKI, R. J. ETTARO, K. M. VEROS, S. D. CLARK;
Univ. At Buffalo - Suny, Buffalo, NY

Abstract: The Neuropeptide S Receptor (NPSR) is a G-protein-coupled receptor implicated in disease states such as post-traumatic stress disorder. Neuropeptide S (NPS), the endogenous ligand to the NPSR, signals through various pathways including the second messengers cAMP and Ca^{2+}. In mice, the central administration of NPS results in hyperlocomotion, increased memory retention, and an anxiolytic phenotype. Compound RTI-263, a truncated form of NPS, has been previously shown in vitro to preferentially signal through the Ca^{2+} second messenger pathway. The central administration of RTI-263 in mice produces similar anxiolytic-like behaviors and improved memory seen with NPS, but a significantly smaller locomotive effect. This exploratory study focused on ascertaining whether the behavioral NPSR-mediated phenotypes in mice translate to rats. Investigating these effects would aid in determining whether the NPSR is a viable drug target to treat anxiety disorders. Male Sprague-Dawley rats were cannulated intracerebroventricularly and assigned to one of three groups: artificial cerebrospinal fluid (aCSF), NPS, or RTI-263 (n = 20 for each group with each study replicated three times). These animals underwent various behavioral and motor tests to determine their levels of locomotion and anxiolytic-like behaviors. Animals were injected with 1 nmole of their assigned compound and tested in a paradigm to determine possible effects of the compound. The same animals were tested in the same paradigm the following day to determine any lasting effects, and animals were not treated with their test compound sooner than 48 hours after the previous injection. Data was analyzed automatically by computer programs or manually by observers unaware of the animals' treatment. Consistent with the findings in mice, NPS-treated rats displayed significantly more locomotion than aCSF or RTI-263 treated animals. Additionally, NPS-treated rats displayed significant anxiolytic-like behaviors in the light dark box paradigm, whereas RTI-263-treated rats trended towards having anxiolytic-like behaviors in the forced swim test. In summary, these results indicate that the NPSR is likely a viable drug target to treat anxiety disorders, as the behavioral phenotypes seen in mice are present in rats.

Disclosures: **A.M. Wojciechowski:** None. **R.J. Ettaro:** None. **K.M. Veros:** None. **S.D. Clark:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.06/B19

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH R01 NS094597
NIH T32 GM067550

Title: Peptide neurotransmitter biosynthesis utilizes cysteine and serine protease pathways possessing distinct peptide dibasic cleavage specificities revealed by global substrate profiling by mass spectrometry

Authors: ***M. C. YOON**, A. O'DONOGHUE, V. HOOK;
UCSD, La Jolla, CA

Abstract: Peptide neurotransmitters, neuropeptides, are required for cell-cell signaling in the nervous system. Active neuropeptides are generated in secretory vesicles from inactive pro-neuropeptide precursors by proteolytic processing at dibasic residue sites (ie., KR, RK, KK, RR). Secretory vesicles utilize the cysteine proteases, cathepsin L (Cat.L) and cathepsin V (Cat.V) and the serine proteases, pro-protein convertases 1 and 2 (PC1/3 and PC2) to process pro-neuropeptides. Gene silencing and gene expression studies have indicated the roles of these proteases in producing enkephalin, beta-endorphin, NPY, cholecystokinin, and other neuropeptides. Differences in cleavage specificities at the dibasic residues have been observed for these proteases for several pro-neuropeptide substrates. Therefore, to test the hypothesis of differential dibasic cleavage properties of proteases involved in pro-neuropeptide processing, this study analyzed protease cleavage properties by global multiplex substrate profiling by mass spectrometry (MSP-MS). MSP-MS utilizes a 228 14-mer peptide substrate library designed to contain the different dibasic sequences as well as all types of protease cleavage sites. Human recombinant Cat.L, Cat.V, PC1/3, and PC2 were incubated with the peptide library in time-course assays and peptide products were identified and quantitated by LC-MS/MS tandem mass spectrometry. Cat.L and Cat.V were found to cleave at the N-terminal side of the dibasic sites and between dibasic residues (-K-R-, -R-K-, and -K-K-). In addition, these enzymes have preference for hydrophobic residues at the P2 position of the cleaved peptide (cleavage between P1-P1’ of P2-P1-P1'-P2' residues). Not all dibasic residue containing substrates were cleaved, suggesting selectivity for peptide substrates. In contrast, the serine proteases PC1/3 and PC2 cleaved at the C-terminal side of dibasic sites such as -R-R- and -K-R, respectively, with only one substrate cleaved by each. Further, PC2 prefers the tribasic processing site, -K-R-R. Results

show: (a) different cleavage specificities at dibasic processing sites for Cat.L and Cat.V compared to PC1/3 and PC2, and (b) protease selectivity for peptide substrates, which implicates different protease roles for processing specific pro-neuropeptides. These newly revealed cleavage specificity properties may facilitate future studies to predict selective protease processing of pro-neuropeptides, and lead to development of selective peptidic inhibitors that may provide novel therapeutic approaches for neuropeptide-related diseases.

Disclosures: **M.C. Yoon:** None. **A. O'Donoghue:** None. **V. Hook:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.07/B20

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: An optimised method to quantify target engagement of Orexin-1 receptors and correlation with biophase free concentration of a selective OX1 receptor antagonist in the rat brain

Authors: R. CARLETTI, A. POFFE, G. AMBROSI, V. MAMMOLI, V. PAVONI, L. PICCOLI, P. CAVALLINI, *L. CABERLOTTO, P. A. GERRARD;
Aptuit, an Evotec Co., Verona, Italy

Abstract: A key part of a drug discovery strategy is to ensure that compounds identified in the screening cascade are able to engage the target under investigation at relevant blood concentrations. Receptor occupancy (RO) measurements, using methods such as *in vivo/ex vivo* autoradiography, have proved extremely useful in identifying novel CNS candidate compounds due to their high translational value.
Orexins are neuromodulatory peptides involved in the control of diverse physiological functions through interaction with two receptors; Orexin-1 and Orexin-2 receptors (OX1-R and OX2-R respectively).
The primary aim of this study was to measure OX1 RO in one specific brain region (tenia tecta) using *ex vivo* binding to rat brain sections, 60 min after the administration of 5 different doses (1, 3, 10, 30 and 60 mg/kg, ip, n=4 per dose group) of the selective OX1 antagonist GSK1059865. Total and free concentrations of GSK1059865 were determined both in blood and brain using mass spectrometry. Receptor autoradiography using the anterior brain portion was performed using the radioligand [^{3}H]-SB674042 to allow determination of RO. Preliminary studies were conducted to optimize the protocol for RO measurements to reduce the risk of compound dissociation and underestimation of RO.
Dose/RO: GSK1059865 dose-dependently occupied OX1-R 60min following ip administration with RO levels reaching maximal RO (>90%) at 60 mg/kg (estimated OD_{50} = 3.9 ± 0.5 mg/kg). A relationship between the compound concentration in blood and brain and the RO at the 5

different doses showed that OX1 RO measured was in very good agreement with the theoretical RO (tRO %) calculated based on the affinity, exposures and free fraction of the compound in the same brains.
Time-course/RO: OX1 RO was also measured at 6 different time points (15min, 30min, 60min, 2hrs, 4hrs and 6hrs, n=4/group) following administration of a single dose (60 mg/kg, ip) of GSK1059865.
Maximal RO levels (>90%) were observed at 30min/60min and relatively high levels were maintained for up to 6hrs (66.4%).
A good PK/RO relationship between measured OX1 RO levels and compound exposures in blood and brain was observed, in line with the relatively fast dissociation kinetics of the compound from OX1-R.
The data indicates that *ex vivo* autoradiography using rat brain sections can be used successfully for OX1 RO studies, since values of RO obtained experimentally were similar to the theoretical RO in the same brain, confirming the sensitivity of the assay.
This study confirms that the OX1 RO assay described provides a robust assay for use in Drug Discovery projects to identify novel OX1-R antagonists targeting the Central Nervous System.

Disclosures: R. Carletti: None. **A. Poffe:** None. **G. Ambrosi:** None. **V. Mammoli:** None. **V. Pavoni:** None. **L. Piccoli:** None. **P. Cavallini:** None. **L. Caberlotto:** None. **P.A. Gerrard:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.08/B21

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH Grant R01-DA034777

Title: Evaluation of novel dual-activity opioid-NPFF ligands for receptor affinity, antinociception and tolerance liabilities

Authors: *J. P. MCLAUGHLIN[1], K. L. MCPHERSON[2,3], M. MOTTINELLI[2], W. SHENG[1], S. O. EANS[1], V. B. JOURNIGAN[4,3], C. MESANGEAU[3], C. R. MCCURDY[2];
[1]Pharmacodynamics, [2]Medicinal Chem., Univ. of Florida, Gainesville, FL; [3]BioMolecular Sci., Univ. of Mississippi, University, MS; [4]Sch. of Pharm., Marshall Univ., Huntington, WV

Abstract: Tolerance limits the analgesic clinical value of mu-opioid receptor (MOR) agonists. Neuropeptide FF (NPFF) mediates hyperalgesia and opioid-induced tolerance through the activation of NPFF-1 and -2 receptors. We hypothesized that ligands with dual MOR agonist/NPFF receptor antagonist activity would produce antinociception with reduced tolerance.

Accordingly, a series of ligands were designed with putative dual opioid and NPFF pharmacophoric elements. Nineteen of these novel ligands were synthesized and screened with competition radioligand binding assays *in vitro*, demonstrating a range of affinity for mu-, kappa-, and delta-opioid receptors (nM) as well as NPFF-1 and -2 receptors (μM). Subsequent *in vivo* screening of all compounds (30 nmol, i.c.v.) in mice with 55ºC and 48ºC warm-water tail-withdrawal assays identified three compounds with better analgesia and anti-hyperalgesia performance, VBJ-192, VBJ-215 and KGM01082. Following up with a more detailed assessment, all three compounds dose-dependently produced equipotent antinociception lasting at least 50 min, with ED50 (and 95%CI) values of 6.9(4.7-9.5), 16(3.5-38.8) and 22.2(11.3-36.6) nmol, icv, respectively that was antagonized by pretreatment with mu- or kappa-opioid receptor antagonists. All three compounds also dose-dependently attenuated NPFF-induced hyperalgesia. Unlike morphine, when tested in the acute antinociceptive tolerance test, repeated dosing of VBJ-215 showed no tolerance, while VBJ-192 and KGM01082 showed moderate tolerance commensurate with their magnitude of NPFF antagonism. In further examination of the three compounds, mice administrated with VBJ-192 or VBJ-215 showed neither respiratory depression nor elevated ambulation in the Comprehensive Lab Animal Monitoring System (CLAMS), and both VBJ-215 and a low dose of VBJ-192 did not impair coordinated locomotor activity on the rotorod (30 and 100 nmol, i.c.v.). Together, these results confirm the mediating effect of NPFF on opioid tolerance, and suggest the potential of dual-action opioid-NPFF ligands as analgesics with fewer liabilities of use.

Disclosures: **J.P. McLaughlin:** None. **K.L. McPherson:** None. **M. Mottinelli:** None. **W. Sheng:** None. **S.O. Eans:** None. **V.B. Journigan:** None. **C. Mesangeau:** None. **C.R. McCurdy:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.09/B22

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH: RCMI MD007600
NIH: NIGMS-RISE R25 GM061838
NSF: CREST HRD-1137725
NSF: PIRE OISE 1545803
NAS: U.S.-Egypt Science and Technology (S&T) Joint Fund 2000007152
NAS: Science and Technology Development Fund (STDF, Egypt): USC17-188

Title: Distribution and expression of the FMRFa-gated sodium channel in the CNS of Biomphalaria glabrata, an intermediate host of Schistosoma mansoni

Authors: *L. C. VICENTE-RODRÍGUEZ[1], S. ROLÓN-MARTÍNEZ[1], P. MÉNDEZ-DEJESÚS[1], M. ROSA-CASILLAS[2], A. HERNÁNDEZ-VÁZQUEZ[2], C. RAMÍREZ-SANTIAGO[2], J. ROSENTHAL[3], M. W. MILLER[1];
[1]Anat. and Neurobio., Univ. of Puerto Rico, Med. Sci. Campus, Inst. of Neurobio., San Juan, PR; [2]Biol., Univ. of Puerto Rico, Río Piedras Campus, San Juan, PR; [3]Marine Biol. Lab., Woods Hole, MA

Abstract: Schistosomiasis is a disease of major concern for health and socioeconomics globally. This neglected tropical disease represents a health burden for more than 70 countries in Africa, Asia, and South America. It is estimated that more than 240 million of people required preventive treatment in 2016, and thousands are at risk of death annually. *Schistosoma mansoni* is the trematode species that causes the most widespread form of intestinal schistosomiasis. The *S. mansoni* life cycle requires two host organisms: snails from the genus *Biomphalaria* as its intermediate host, and mammals as its definitive host. Snails undergo major physiological and behavioral changes upon parasitic infection, but the neural contribution to these changes is poorly understood. FMRFamide neuropeptides regulate diverse physiological processes in the snail that are known to be affected by parasitosis, such as respiration, reproduction and feeding.We hypothesized that the FMRFamide peptide system participates in the behavioral and physiological modifications that occur in the snail following the parasitic infection. As a first step toward testing this hypothesis, we identified a FMRFamide-gated sodium channel (*FaNaC*) in neural transcriptomes from two *Biomphalaria* species, *B. glabrata* and *B. alexandrina* (Mansour et al. 2017). The *FaNaC* is unique to this phyla and the only known sodium channel gated by a peptide**.** We performed immunohistochemistry and western blot experiments in snail CNS samples to determine the *FaNaC* receptor distribution and expression. We found a change in the neuronal distribution and expression of this receptor in infected snails. FMRFamide signaling, specifically through its ionotropic *FaNaC* receptor, may participate in in the physiological changes observed in *B. glabrata* during parasitic infection. As this receptor is unique to molluscs, it may also provide an effective and selective target for controlling *B. glabrata* proliferation.

Disclosures: L.C. Vicente-Rodríguez: None. **S. Rolón-Martínez:** None. **P. Méndez-DeJesús:** None. **M. Rosa-Casillas:** None. **A. Hernández-Vázquez:** None. **C. Ramírez-Santiago:** None. **J. Rosenthal:** None. **M.W. Miller:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.10/B23

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: The Scientific and Technical Research Council of Turkey (TUBITAK - Project number: 2015S699).

Title: Chemoarchitecture of the human brainstem

Authors: *G. SENGUL[1], U. TURE[2], G. PAXINOS[3];
[1]Ege Univ. Sch. Med., Izmir, Turkey; [2]Neurosurg., Yeditepe University, Sch. of Med., Istanbul, Turkey; [3]Neurosci. Res. Australia, Randwick, Australia

Abstract: Brainstem is of great significance being responsible in vital functions such as respiration, locomotion, arousal as well as cognition and affection. Nuclei in brainstem have been related to important and widespread diseases such as progressive supranuclear palsy, multiple system atrophy, otism spectrum disorders and Parkinson's disease. In this study, the the neurochemical content of the human brainstem was investigated using histochemical and immunohistochemical stainings. Brainstem sections were cut using a cryostat at a thickness of 60 micrometers and stained using Nissl, Weigert and NADPH-diaphorase immunohistochemical stains, and numerous immunohistochemical markers such as calbndin, calretinin, calcitonin-gene related peptide, parvalbumin, glycine, GAD67, choline acetyltransferase, enkephalin, neurofilament-SMI 32, neuronal nuclear protein (NeuN), glutamate and cocaine amfetamine related peptide. Based on these stainings, a map of the human brainstem was created to be a major reference to both basic science researchers and clinicians, and also be a reference for radiological imaging techniques.

Disclosures: G. Sengul: None. **U. Ture:** None. **G. Paxinos:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.11/B24

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Novel treatments to opiate dependence - A review

Authors: S. S. VENKATARAMAN, ***N. DAFNY**;
Neurobio. and Anat., The Univ. of Texas McGovern Med. Sch. at Houston, Houston, TX

Abstract: Repetitive use of opioids results in dependence on the drug, a complex condition that is considered to be a substance use disorder. The reduction or cessation of opioid consumption leads to severe withdrawal behaviors. The degree of opiate dependence can be assessed by the intensity of the withdrawal behavior. To prevent these devastating experiences, the subject will continue to take the drug. Success in modifying the withdrawal behavior may shed light on the dynamics of opiate dependence and the opioid epidemic. Reducing the severity of the withdrawal

symptoms is the prime treatment of opioid dependence. Although several pharmacological treatment options are available to attenuate the symptoms of opioid withdrawal, the effectiveness of them is limited. Classical therapeutic addiction research has focused on cellular and molecular alterations within neurons and their neuronal circuits. As such, most of the pharmacotherapies for opioid addiction are designed to target the neuronal processes known to be affected by drug intake. In addition to the pivotal role of neurons in the initiation, transition, and maintenance of opioid addiction, the glial cells within the central nervous system are also of particular importance. According to some studies, 60 to 80% of the cellular brain is composed of glial cells. Recent studies have shown that glial cells participate in synaptogenesis, neuronal excitability, and neurotransmission. Following opioid exposure, glial cells demonstrate robust changes in their morphology and physiology in key central nervous system regions known to contribute to drug dependence. They play a pivotal role in opioid-addiction like behaviors. Glial cells are also part of the immune system. This review presents preclinical studies demonstrating that the immune system participates in the expression of opiate withdrawal and that a single dose of immunological substances such as α-interferon, cyclosporine, and cortisol significantly attenuate the severity of the naloxone-induced withdrawal symptoms in opioid-dependent animals. We hope that this review will encourage clinical studies to use immunomodulators in combating the opioid epidemic and save lives.

Disclosures: S.S. Venkataraman: None. **N. Dafny:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.12/B25

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH: RCMI MD007600
NIH: MBRS GM087200
NSF: DBI-1337284
NSF: HRD-1137725
OISE 1545803
National Academy of Sciences (NAS; USA): U.S.-Egypt Science and Technology (S&T) Joint Fund 2000007152
Science and Technology Development Fund (STDF, Egypt): USC17-188.

Title: Localization of adipokinetic-like immunoreactivity in the central nervous system of Biomphalaria ssp., intermediate hosts for schistosomiasis

Authors: *M. ROSA CASILLAS[1], P. MÉNDEZ DE JESUS[2], M. R. HABIB[3], R. P. CROLL[4], M. W. MILLER[2];

[1]Biol., Univ. of Puerto Rico, Rio Piedras, San Juan, Puerto Rico; [2]Inst. of Neurobio., San Juan, PR; [3]Theodor Bilharz Res. Inst., Imbaba, Egypt; [4]Dalhousie Univ., Halifax, NS, Canada

Abstract: Approximately 200 million people live at risk of contracting the parasitic disease schistosomiasis. The digenetic trematode species *Schistosoma mansoni*, which causes the most common form of intestinal schistosomiasis, requires freshwater snails from the genus *Biomphalaria* to serve as its primary intermediate host. Within the snail, *S. mansoni* larvae multiply and transform into cercariae that can infect humans. As infection by trematode parasites can alter neuropeptide expression in snail hosts, a neural transcriptomics approach was undertaken to explore the neuropeptidome of *Biomphalaria glabrata*, the major intermediate host in the Western Hemisphere, and *Biomphalaria alexandrina*, the principal intermediate host in Egypt. A *B. alexandrina* transcript (4,038 nucleotides) encoded a precursor prohormone, from which a single adipokinetic hormone (AKH) related neuropeptide could be liberated. For this investigation, an antiserum (rabbit polyclonal) generated against pQIHFTPDWGNNamide, was used to localize AKH-like immunoreactivity in the central nervous system (CNS) of *B. glabrata.* In this species, a symmetrical cluster of 5 ± 0.8 and 5 ± 1.1 medium sized (20-30 μm) cells as well as two pairs of distinct, single neurons, were present in the right and left cerebral ganglia, respectively. Also, a single, large (30-40 μm) cell body was present in the visceral ganglion. These cells appeared to give rise to fiber tracts projecting throughout the whole CNS, particularly into the right parietal nerves, as well as the visceral ganglion which ultimately projects into the intestinal and anal nerves. As in other invertebrate systems, signaling by neurons containing an AKH-like neuropeptide could regulate stress responses during the course of infection in this host-parasite system.

Disclosures: M. Rosa Casillas: None. **P. Méndez De Jesus:** None. **M.R. Habib:** None. **R.P. Croll:** None. **M.W. Miller:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.13/B26

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: RUSH-IMSD (Initiative to Maximize Student Development) R25 109421
R01NS060632-10

Title: Beta-catenin negatively regulates IL-6 and IL-8 expression at the transcriptional level and induces reactivity in human astrocytes

Authors: *K. ROBINSON, S. NARASIPURA, L. AL-HARTHI;
Microbial Pathogens and Immunity, Rush Univ. Med. Ctr., Chicago, IL

Abstract: HIV invades the brain during acute infection, setting the stage for persistent neuroinflammation despite combined antiretroviral therapy (cART) and leads to HIV-Associated Neurocognitive Disorders (HAND), which occurs in ~50% of HIV-infected individuals. Our lab is focused on understanding the role of Wnt/β-catenin signaling in HAND. Here, we evaluated the impact of β-catenin on inflammatory mediators associated with neuroinflammation, chemotactic molecules, and regulation of A1 (proinflammatory)/A2 (protective/repair) phenotypes of astrocytes. We demonstrate that knockdown (KD) of β-catenin in normal human astrocytes (NHAs) significantly induced IL-6 and IL-8 at the transcription and protein levels and conversely, induction of β-catenin significantly downregulated these two molecules. These findings are intriguing given that no role for β-catenin to date is associated with IL-6 and IL-8 regulation. Further, KD of β-catenin induced three genes associated with A1 phenotype by 2.4-6.4 fold. These findings indicate that β-catenin expression in astrocytes is a critical regulator of anti-inflammatory responses and its disruption can potentially mediate persistent neuroinflammation.
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Disclosures: K. Robinson: None. **S. Narasipura:** None. **L. Al-Harthi:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.14/B27

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Nishinomiya Basic Reserch Fund, Japan

Title: Elucidation of the regulatory mechanisms of peripheral axon outgrowth by Wnt5a, released from mechanically stimulated rat periodontal ligament cells

Authors: *K. TAKAHASHI, T. YOSHIDA, M. WAKAMORI;
Tohoku University, Grad. Sch. of Dent., Sendai, Japan

Abstract: Mechanical stimuli such as extension and compression associated with the movement and the gravity influence biological activities of all cells. Recent studies have shown that mechanical stimuli give rise to intracellular biochemical signaling cascades through mechanosensors and mechanotransducers. The periodontal ligaments (PDLs), located at the interface between the tooth and alveolar bones, are responsible for tooth planting and pressure absorbing actions. In addition, the Ruffini's corpuscles, one of the mechanoreceptors located in the PDLs, play a pivotal role in pressure sensing to identify various food properties and to adjust occlusal force. Although recent studies have shown that branches of Ruffini's corpuscles are not formed without mechanical stimulation in the rat PDL, there is no report to assess the regulatory

mechanisms for the peripheral axonal structure by mechanically stimulated PDL cells.
We have established primary PDL cell (rPDL)lines derived from rat PDLs. The RT-PCR analysis confirmed the expression of NGF, BDNF, neurotrophin-4 (NT-4) and Wnt5a mRNA in the rPDL cells. The rPDL cells were seeded on silicon chamber and loaded with periodic mechanical stimulation (0.5 Hz, 15% expansion). The qPCR analysis revealed the expression level of Wnt5a mRNA in rPDL cells increased in a stimulation-period dependent manner, while that of neurotrophic factors did not. LY294002, a PI3K inhibitor, and U0126, a MEK1/2 inhibitor, diminished the increase of Wnt5a mRNA expression level in the rPDL cells loaded with mechanical stimulation. In order to confirm whether the released Wnt5a can elongate the neurite, the culture media for the primary mouse trigeminal ganglion neurons were replaced with the supernatant media of the rPDL cells with or without mechanical stimulation. The supernatant media of the mechanically stimulated rPDL cells enhanced the neurite elongation and this effect was suppressed by anti-Wnt5a antibody.
These results suggest that the mechanical stimulated PDL cells produce Wnt5a via MEK1/2 and/or PI3K pathways and the secreted Wnt5a from PDL cells elongates neurites.

Disclosures: **K. Takahashi:** None. **T. Yoshida:** None. **M. Wakamori:** None.

Poster

033. Opiates, Cyotokines, and Other Neuropeptides

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 033.15/B28

Topic: B.06. Synaptic Transmission

Support: NMRC/CBRG/0041/2013
NUS Research Scholarship

Title: Investigation of Hippocampal Area CA2 Dependent Behavioural Metaplasticity in Adult Rats Affected by Juvenile Stress

Authors: ***R. RAGHURAMAN**[1], S. SAJIKUMAR[2];
[2]Physiol., [1]Natl. Univ. of Singapore, Singapore, Singapore

Abstract: In the recent years, the implication of social memory has been well established in the CA2 area of hippocampus. With the aim of exploring social interaction and sociability in rats that were subjected to juvenile stress, we address questions of if and how the neural circuitry is altered, and thereby its aftermath in social behaviour. Our preliminary results have brought about pivotal insights of how a juvenile stressed rat shows lower sociability yet a higher social interaction with a never-before-met stranger rat. Upon further probing the electrophysiological properties in the area CA2, LTP resistance was observed not just in the SC-CA2 (indirect) synapses as has been shown in the healthy rodents, but also in the EC-CA2 (direct) synapses. It

has been established very recently, that (the neuropeptide, Substance-P) SP-induced potentiation of SC-CA2 synapses transforms a short-term potentiation of EC-CA2 synaptic transmission into long term potentiation, consistent with synaptic tagging and capture hypothesis, also proving that this associative interaction between both the inputs is independent of GABAergic system. However, in the case of juvenile stressed rats, contrary to the above finding, an exogenous bath application of SP did not result in long term potentiation showing occlusion of LTP. Delineating the molecular signature using staining and biochemical analyses in this area that renders the said electrophysiological properties and thereby behaviour, by employing selective antagonist (L733-060), helped us further understand that memory mechanisms in area CA2 is regulated through NK1 receptors under juvenile stressed conditions.

Disclosures: R. Raghuraman: None. **S. Sajikumar:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.01/B29

Topic: B.02. Ligand-Gated Ion Channels

Title: Electrophysiological characterization of recombinant NMDA (GluN1/2A, GluN1/2B, GluN1/2C and GluN1/2D) and AMPA (GluA2) cell lines using an automated HTS electrophysiology platform

Authors: P. MADAU, L. HUTCHISON, A. DICKSON, C. KADI, L. MCCRACKEN, H. TRACEY, D. SMITH, C. BROWN, L. GERRARD, ***D. DALRYMPLE**, I. MCPHEE, D. PAU; SB Drug Discovery, Glasgow, United Kingdom

Abstract: The ionotropic Glutamate receptors comprise members of the NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid) and Kainate receptor classes. These important excitatory channels are mainly located in the central nervous system (CNS), but also in peripheral locations such as sensory nerve terminals in skin and cardiac ganglia. NMDA and AMPA receptors have been targets for the development of new drugs to treat a series of neurological and neurodegenerative disorders, including neuropathic pain and Parkinson's disease, and play a key role in synaptic plasticity, memory and learning. Drugs that target the neurotransmitter systems are immensely important as therapeutic agents and as research tools for scientists seeking to unravel the complexities of neuronal signalling. Developments in HTS technologies have allowed rapid assessment of large numbers of compounds against ion channel drug targets using patch-clamp electrophysiology. In conjunction with these technologies, robust high-quality recombinant cell lines are essential when testing these fast-activating Glutamatergic NMDA and AMPA receptors.
We have successfully developed four recombinant NMDA (GluN1/2A, GluN1/2B, GluN1/2C

and GluN1/2D) and one AMPA (GluA2) receptor cell lines using stably transfected, inducible HEK cells. Validation of these cell lines was performed using high-throughput automated electrophysiology which allows the testing of multiple cell lines in a single experiment. This system enables rapid drug application resulting in high-quality data that is reproducible over multiple experiments. Concentration-response curves (EC_{50} and IC_{50}) on NMDA and AMPA channels of both agonists NMDA (+Glycine) and Glutamate and antagonists Ketamine and Cyanquixaline (CNQX) were consistently obtained and correlated well with literature values. Further assays were developed for both NMDA and AMPA receptors in order to detect changes in activity using different levels of glutamate activation induced by both unknown (e.g. novel agents), classical positive (e.g. Pregnenolone sulphate and Cyclothiazide) and negative (e.g. TCN 201 and CNQX) modulators in a single experiment using multiple cell lines.
Development of rapid and robust assays for these targets enables HTS screening against a panel of Glutamatergic subunit combinations, generating a wealth of high-quality electrophysiology data. This should allow researchers to generate more selective NMDA and AMPA receptor-targeted drugs to improve their efficacy, safety and tolerability for a wide range of therapeutic purposes.

Disclosures: **D. Dalrymple:** None. **P. Madau:** None. **L. Hutchison:** None. **A. Dickson:** None. **C. Kadi:** None. **L. McCracken:** None. **H. Tracey:** None. **D. Smith:** None. **C. Brown:** None. **L. Gerrard:** None. **I. McPhee:** None. **D. Pau:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.02/B30

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01MH116003
Yerkes Primate Center NIH/ORIP base grant P51OD011132

Title: Ultrastructural localization of glutamate receptor delta-1 subunit in the mouse striatum

Authors: A. HOOVER[1,2], R. M. VILLALBA[1,2], *J.-F. PARE[1,2], J. LIU[3], P. J. GANDHI[3], G. P. SHELKAR[3], D. M. SHASHANK[3], Y. SMITH[1,2,4];
[1]Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [2]Udall Ctr. of Excellence for Parkinson's Dis., Atlanta, GA; [3]Pharmacol. and Neurosci., Creighton Univ. Sch. of Med., Omaha, NE; [4]Neurol., Emory Univ., Atlanta, GA

Abstract: The glutamate delta-1 (GluD1) receptor is an ionotropic glutamate receptor (iGluR) found throughout the hippocampus, cortex, cerebellum, striatum, and central nucleus of the amygdala. In contrast to other iGluRs, GluD1 does not exhibit the typical ligand-gated fast ion

flow. It rather serves as a synaptic organizer that mediates the formation and/or maintenance of synapses via trans-synaptic interaction with Cerebellin1/2 (Cbln1/2)-Neurexin1 complex. GluD2 found in the cerebellum is the more heavily studied relative of GluD1, which is responsible for the maintenance and regeneration of parallel fiber-Purkinje cell synapses. In contrast to GluD2, the precise contribution of GluD1 to the development or maintenance of glutamatergic synapses in the forebrain remains poorly understood. GluD1 is enriched in the striatum, a region that governs motor, cognitive and reward behaviors. The striatum receives strong excitatory inputs from the cerebral cortex and thalamus which express several members of Cbln family proteins. To gain a better understanding of the potential sites of striatal GluD1 function, we used electron microscopy immunohistochemistry to map the cellular and subcellular localization of GluD1 immunoreactivity in the mouse dorsal striatum (N=3) using a highly specific GluD1 antibody. At the light microscopic level, GluD1 immunostaining was homogeneously distributed throughout the whole striatal neuropil. In the electron microscope, ~50% GluD1-labeled elements were categorized as dendritic shafts, 40% as glial cell processes and ~10-15% as dendritic spines. In some instances, the GluD1 immunoreactivity was aggregated at the post-synaptic densities of glutamatergic synapses. Immunogold studies are in progress to further define the subsynaptic localization of GluD1 in relation to different glutamatergic synapses. Our preliminary data suggest that striatal GluD1 is located to subserve regulatory effects upon specific glutamatergic synapses and glial function in the mammalian striatum.

Disclosures: **A. Hoover:** None. **R.M. Villalba:** None. **J. Pare:** None. **J. Liu:** None. **P.J. Gandhi:** None. **G.P. Shelkar:** None. **D.M. Shashank:** None. **Y. Smith:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.03/B31

Topic: B.02. Ligand-Gated Ion Channels

Support: NSF 1456818

Title: Differential role of GluD1 in synapse maintenance

Authors: *P. J. GANDHI, J. LIU, G. SHELKAR, R. PAVULURI, S. M. DRAVID; Pharmacol. and Neurosci., Creighton Univ. Sch. Of Med., Omaha, NE

Abstract: The delta family of glutamate receptor (GluD1, GluD2) belong to a class of ionotropic glutamate receptor (iGluR), forms synapses by binding to its presynaptic partner neurexin (NrX) via synaptogenic protein cerebellin1 (cbln1). It is known that GluD1 is widely expressed in adult mouse brain with rich expression in cerebral cortex, striatum and limbic regions like nucleus accumbens and hippocampus. However, its role in maintenance of synaptic function remains

largely elusive. We performed whole cell patch clamp recordings from medium spiny neurons (MSNs) of dorsal striatum and nucleus accumbens as well as from pyramidal neurons of medial prefrontal cortex (mPFC, layer II/III) and hippocampal CA1. GluD1 deletion from dorsal striatum led to significant reduction in mEPSC frequency but not amplitude and reduced excitability. We did not observe any changes in inhibitory neurotransmission as well as mGlur1/5 mediated long-term depression (LTD). Ablation of GluD1 from mPFC increased mEPSC frequency with no change in amplitude. Whereas loss of GluD1 from nucleus accumbens and hippocampus did not affect basal excitatory neurotransmission and plasticity. These results suggest that GluD1 in cortex, hippocampus and nucleus accumbens is not obligatory or homeostatic mechanisms compensate for its loss. However, absence of GluD1 from dorsal striatum significantly reduced excitatory neurotransmission. In ongoing neuroanatomical studies, we are assessing changes in glutamatergic inputs upon GluD1 deletion in dorsal striatum using presynaptic glutamatergic markers vGlut1 and vGlut2 and analysis of spine density and morphology. Together, our findings suggest that in dorsal striatum, GluD1 is essential for excitatory synapse maintenance.

Disclosures: **P.J. Gandhi:** None. **J. Liu:** None. **G. Shelkar:** None. **R. Pavuluri:** None. **S.M. Dravid:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.04/B32

Topic: B.02. Ligand-Gated Ion Channels

Support: NSF1456818
NIH R01MH116003-01A1

Title: Effect of region specific deletion of glutamate delta 1 receptors on cognitive flexibility in mice

Authors: D. Y. GAWANDE, G. P. SHELKAR, R. PAVULURI, *S. M. DRAVID;
Pharmacol. and Neurosci., Creighton Univ., Omaha, NE

Abstract: Glutamate delta1 (GluD1) receptors belong to ionotropic glutamate receptor family, but do not function as a conventional ligand-gated ion channel. Instead it possesses a unique synaptogenic function by interacting with its presynaptic partner neurexin 1 (Nrxn1) via synaptogenic protein cerebellin 1. Previous reports suggest an enriched expression of GluD1 in major brain regions like, hippocampus, cortex, thalamus and striatum. Interestingly these regions are involved in execution of higher order functions like learning and memory. We have recently reported the potential role of GluD1 in cocaine addiction. However the effect of region specific

ablation of GluD1 and their effect on cognitive flexibility remains unclear. Therefore in present study we used combination of constitutive and conditional GluD1 KO mouse models to evaluate the region specific effect of GluD1 receptors on cognition. We found the selective deletion of GluD1 from striatum showed pronounced repetitive behavior in water T-maze test. Whereas cortical and hippocampal GluD1 receptors deletion did not affect behavioral flexibility. In constitutive KO mice we found significant deficit in water T-maze learning as well as reversal learning compared to the wildtype mice. Together, these findings demonstrate a critical role of striatal GluD1 in maintaining the cognitive flexibility in mice.

Disclosures: D.Y. Gawande: None. **G.P. Shelkar:** None. **R. Pavuluri:** None. **S.M. Dravid:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.05/B33

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01 N5078792
NIH Grant R01 AG055357

Title: Regulation of AMPAR membrane insertion by beta2-adrenergic receptor-Cav1.2 interaction

Authors: *K. KIM, D. GHOSH, B. LEE, M. NAVEDO, J. HELL;
Pharmacol., Univ. of California Davis, Davis, CA

Abstract: Postsynaptic AMPA-type glutamate receptors (AMPARs) mediate most of the synaptic transmission at the excitatory synapses which constitute ~80% of a mammalian brain. Synaptic strength mostly depends on the abundance of AMPARs at the postsynaptic site, and dynamic nature of AMPARs according to the neuronal activity is a basis of synaptic plasticity, a molecular mechanism of learning and memory. The β2 adrenergic receptor (β2AR) mediates the effect of norepinephrine (NE) which is important for attention and learning. It forms two unique signaling complexes with AMPARs and L-type voltage-gated Ca^{2+} channel Cav1.2, a main Ca^{2+} source for neuronal excitation/transcription coupling, LTP and learning. When activated, β2AR induces phosphorylation of AMPAR and Cav1.2 by PKA, which in turn results in an increase in membrane insertion and Ca^{2+} influx, respectively, of each channel. It was demonstrated that Ca^{2+} influx from Cav1.2 stabilizes surface AMPARs. In this study, I investigated if the correct localization of Cav1.2 within a signaling complex with β2AR is important for AMPAR stability. Using extracellular N-terminal fusion of superecliptic-pHluorin (SEP) tag, which is fluorescent at pH 7.4 (extracellular) but nonfluorescent at pH < 6.0 (endosomal lumen) to differentiate the

surface and cytosolic receptor, membrane insertion of GluA1-containing AMPAR was monitored based on different types of behavior: 1) stable insertion, 2) transient insertion, and 3) full fusion. When dissociated hippocampal neurons expressing SEP-GluA1 (and untagged GluA2) are treated with NE to stimulate β2AR, all types of GluA1 membrane insertion were increased. Interestingly, incubation of neurons with a peptide breaking the interaction between β2AR and Cav1.2 greatly decrease all types of GluA1 membrane insertion. These results indicate that the local Ca^{2+} increase by Cav1.2 regulates membrane stability of AMPARs in the vicinity of them, showing the importance of the proper composition of the signaling complex.

Disclosures: K. Kim: None. **D. Ghosh:** None. **B. Lee:** None. **M. Navedo:** None. **J. Hell:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.06/B34

Topic: B.02. Ligand-Gated Ion Channels

Support: Whitehall Foundation

Title: SynDIG4/Prrt1 establishes a reserve pool of GluA1-containing AMPARs required for LTP

Authors: K. E. PLAMBECK[1], *E. DIAZ[2];
[1]Pharmacol., Univ. of California Davis, Davis, CA; [2]Pharmacol., UC Davis Sch. of Med., Davis, CA

Abstract: Regulation of AMPA-type glutamate receptors (AMPARs) at synapses is a predominant mechanism for regulating synaptic strength underlying learning and memory. We identified the transmembrane protein Synapse Differentiation Induced Gene 1 (SynDIG1; SD1) as an AMPAR auxiliary protein that regulates excitatory synaptic strength and AMPAR number both *in vitro* and *in vivo.* The related protein SynDIG4 (SD4; also known as Prrt1) was identified in several independent proteomic screens in complex with AMPARs, suggesting it may also function as an AMPAR auxiliary factor. We showed that some SD4 co-localizes with extra-synaptic GluA1-containing AMPARs in primary neurons, while loss of SD4 results in reduced extra-synaptic AMPARs, implying a role of SD4 outside the synapse. Furthermore, single-tetanus induced long term potentiation (LTP), which is dependent on GluA1, is abolished in hippocampal slices from SD4 knockout (KO) mice. We hypothesize that SD4 maintains an extra-synaptic reserve pool of GluA1-containing AMPARs required for LTP.
Here we show that co-expression of SD4 with GluA1 or GluA2 homomeric AMPARs in COS cells leads to a 50% or 33% increase in mean area of surface AMPAR puncta, respectively. In contrast, SD4 co-expressed with the kainite-type glutamate receptor subunit GluK2 does not change the size of surface GluK2 puncta, indicating SD4 specifically affects surface AMPARs in

COS cells. There is also increased co-localization of GluA1 with SD4 compared to GluK2, consistent with our previous studies indicating preferential localization of SD4 with extra-synaptic GluA1 in neurons.
In hippocampal neurons at baseline, there is a 33% decrease in extra-synaptic surface GluA1 density and puncta size, and a 50% decrease in surface puncta integrated density in SD4 KO hippocampal neurons compared to wild-type (WT), indicating loss of SD4 results in the reduction of a reserve pool of surface GluA1-containing AMPARs. To study GluA1-containing AMPARs during LTP, we used glycine induced chemical LTP (chemLTP). After chemLTP, WT neurons show a 2-fold increase in synaptic GluA1, while synaptic GluA1 is unchanged in SD4 KO neurons. This impairment is rescued by transfecting KO neurons with WT SD4 to restore the reserve pool of extra-synaptic surface GluA1, suggesting the effect is a direct result of the loss of SD4. Lastly, we observed a 50% increase in synaptic SD4 density upon chemLTP, suggesting that SD4 is targeted to synapses along with GluA1 during LTP. Given that LTP requires a reserve pool of AMPARs, these data are consistent with a model whereby SD4 establishes an extra-synaptic reserve pool of GluA1-containing AMPARs required for LTP.

Disclosures: **K.E. Plambeck:** None. **E. Diaz:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.07/B35

Topic: B.02. Ligand-Gated Ion Channels

Support: Whitehall Foundation #2017-08-30
R21MH116315
R01MH117130
RO1NS062736

Title: Differential regulation of apical and basal synapses by serine racemase

Authors: ***S. A. JAMI**[1], J. M. WONG[1,2], D. K. PARK[1], E. V. BARRAGAN[1,2], K. ZITO[1], J. A. GRAY[1,3];
[1]Ctr. For Neurosci., [2]Neurosci. Grad. Group, [3]Dept. of Neurol., Univ. of California, Davis, Davis, CA

Abstract: NMDA receptors (NMDARs) are glutamate-gated ion channels which uniquely require a co-agonist for activation: either glycine or D-serine. The identity of the co-agonist is developmentally regulated and spatially restricted in the brain, with many synapses using glycine early on and later switching to D-serine. Despite the importance of these co-agonists in regulating excitatory synaptic transmission, neuronal function, and synaptic plasticity, the source

and regulation of D-serine remains controversial. Although D-serine and its biosynthetic enzyme serine racemase (SR) were originally thought to be localized in astrocytes, recent studies using SR constitutive & conditional knock-out mice combined with more selective antibodies instead suggest a predominantly neuronal localization. Indeed, we have recently shown that postsynaptic SR regulates NMDA receptor function in a cell-autonomous manner. Here we show that SR deletion has differential effects on NMDA receptor function at synapses on basal versus apical dendrites. Using single-neuron deletion of SR, we found that NMDAR-EPSCs are reduced in both apical and basal synapses on CA1 neurons in adult mice, though AMPAR-EPSCs are reduced only at basal synapses due to a selective loss of basal synapses. Interestingly, in SRKO mice, LTP is impaired only at basal synapses in young mice, but impaired at both basal and apical synapses in adult mice. Overall, these results suggest synapse-specific modes of NMDAR co-agonist regulation.

Disclosures: S.A. Jami: None. **J.M. Wong:** None. **D.K. Park:** None. **E.V. Barragan:** None. **K. Zito:** None. **J.A. Gray:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.08/B36

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NINDS 1R01NS105502-01A1

Title: A mouse model of a neurodevelopmental disorder of a gain-of-function mutation in the GRIK2 kainate receptor gene

Authors: *T. NOMURA[1], E. BINELLI[2], K. WATRAL[2], S. TANIGUCHI[2], J. R. STOLZ[2], G. T. SWANSON[2], A. CONTRACTOR[3];
[1]Physiol., [2]Pharmacol., Northwestern Univ., Chicago, IL; [3]Physiol., Northwestern Univ. Dept. of Physiol., Chicago, IL

Abstract: Kainate receptors (KARs) play diverse roles in the central nervous system and their dysfunction has been associated with the pathophysiology of neurodevelopmental disorders. A recent genetic study identified a gain-of-function mutation in the GRIK2 gene, which encodes for the GluK2 subunit of KARs, in an individual with ataxia, motor and speech delay, and intellectual disability. The mutation results in a threonine substitution for alanine at position 657 (A657T). This amino acid is highly conserved among ionotropic glutamate receptors and is within the gating region of the receptor. *In vitro* analyses of GluK2(A657T)-containing kainate receptors have found that this mutation resulted in a constitutive activation of the receptors that is similar to the gating alteration found in GluD2 with the “Lurcher” A657T mutation. In order to

determine how this mutation affects synaptic and circuit development in the brain we generated the GluK2(A657T) knock-in mouse line using CRISPR/Cas9 gene editing. We found that homozygous mice are not viable but heterozygous mice display early onset ataxia with otherwise grossly normal development. Behavioral tests were performed on wildtype and heterozygous littermates in order to test motor function, social interaction, anxiety, and perseverative behaviors. Juvenile GluK2(A657T) het mice performed significantly worse in tests of motor coordination when compared to wildtype littermates, and did not exhibit typical behaviors such as nestlet shredding and digging. In contrast, het mice showed no differences in open field and zero maze tests suggesting no alterations in anxiety caused by the mutation. To determine the functional effect of this mutation on synaptic transmission and synaptic development we performed electrophysiological recording from CA3 pyramidal cells and analyzed mossy fiber synapses where KARs are synaptically localized. The KAR mediated component of mossy fiber EPSCs in CA3 neurons of GluK2(A657T) het mice decayed with a significantly slower time course compared to recordings from wildtype littermates. In contrast, there was no effect on the relative amplitude of the KAR mediated EPSCs suggesting that assembly, trafficking and synaptic localization of the receptors are not largely affected by the mutation. The prolonged decay of the KAR current in these mice suggests that synaptic summation and integration of mossy fiber EPSCs are altered, which could affect plasticity induction in the CA3 region of the hippocampus. Ongoing experiments are analyzing how mutant KARs affect the development of synapses in the CA3 and whether this alters function of the CA3 microcircuit.

Disclosures: **T. Nomura:** None. **E. Binelli:** None. **K. Watral:** None. **S. Taniguchi:** None. **J.R. Stolz:** None. **G.T. Swanson:** None. **A. Contractor:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.09/B37

Topic: B.02. Ligand-Gated Ion Channels

Support: NIG Grant NS105502

Title: Hotspot variants in the GRIK2 kainate receptor gene are causative for neurodevelopmental disorders with diverse phenotypes

Authors: J. R. STOLZ[1], G. L. CARVILL[2], B. KEREN[3], E. KIRK[4], P. R. MARK[5], C. MIGNOT[3], L. ROHT[6], Z. STARK[7], *G. T. SWANSON[1];
[1]Pharmacol., [2]Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [3]Dept. de Génétique, Ctr. de Référence des Déficiences Intellectuelles de Causes Rares, Hôpital de la Pitié-Salpêtrière, Paris, France; [4]Ctr. for Clin. Genet., Prince of Wales Hosp., Randwick, Australia;

[5]Spectrum Hlth. Med. Genet., Grand Rapids, MI; [6]Tartu Univ. Hosp., Tallinn, Estonia; [7]Ctr. for Clin. Genet., Murdoch Children's Res. Inst., Parkville, Australia

Abstract: Missense variants in several ionotropic glutamate receptor (iGluR) genes have been shown to cause autism and related neurodevelopmental disorders (NDDs). We previously reported the first missense variant in a gene encoding a kainate receptor (KAR) subunit subtype, *GRIK2* (Glutamate receptor, ionotropic, kainate 2), which produced an A657T substitution in the GluK2 subunit (Guzmán et al., 2017). This *de novo* variant was determined to be causative for a non-syndromic NDD with intellectual disability, speech deficits and ataxia as primary clinical features. The A657T substitution occurs in the M3 pore-forming membrane domain, which has been characterized as a "hotspot" of NDD-causing mutations in other iGluR subunit genes. The biophysical properties of GluK2(A657T) subunit-containing receptors were different from wildtype GluK2, as they exhibited greatly enhanced sensitivity to glutamate, slowed decay kinetics and destabilization of the desensitized state of the receptor.
This study prompted us to determine if there are other children with NDDs arising from hotspot mutations in the GRIK2 gene. Whole exome sequencing and informatics analysis identified four additional children with *de novo GRIK2* variants that occur in the exon encoding the M3 and M3-S2 domains. Three children with severe pediatric epilepsy or white matter abnormalities harbored a nonsynonymous missense mutation resulting in a threonine to lysine variation at position 660 (GluK2(T660K)). We also identified an autistic child harboring a missense mutation that resulted in a GluK2(I668T) variant. Introduction of the analogous mutations into recombinant GluK2 subunits had varied effects on the membrane localization and biophysical properties of the receptors when expressed in HEK293 cells. GluK2(T660K) receptors were very similar to GluK2(A657T) in their slowed decay kinetics and destabilized desensitized states, whereas GluK2(I668T) had profoundly reduced peak amplitudes and rapid desensitization. Our data further demonstrate that that variants in the *GRIK2* KAR subunit gene leading to M3/M3-S2 hotspot substitutions alter channel function and in some children cause severe NDDs. A trio of children containing the same variant (T660K) exhibit grossly similar developmental and neurological phenotypes, suggestive of strong penetrance. Dysfunctional KARs are therefore one of the growing list of synaptic signaling molecules that can underlie disorders of brain development.

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Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.10/B38

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01MH053608

Title: Protein interactions of schizophrenia risk genes: Arc-NMDAR complex formation

Authors: *I. M. GONZALEZ, L. YANG, W. ZHANG, P. WORLEY;
Johns Hopkins Med. Univ., Baltimore, MD

Abstract: Activity regulated cytoskeleton-associated protein (Arc, aka Arg3.1) is an immediate early gene product that is critical for dendritic spine regulation and neuronal plasticity. Interestingly, Arc along with NMDA receptors (NMDAR), were identified as "hubs" of protein interaction in screens for *de novo* mutations linked to schizophrenia. Previous studies from our lab revealed that Arc binds NMDAR subunits NR2A and NR2B, suggesting these proteins may form a "superhub" relevant to schizophrenia (Zhang et al., 2015). Here, we examine Arc-NMDAR binding. NR2A contains three Arc binding motifs: aa 1047-1053, aa 1169-1174, and aa 1220-1226. The NR2B subunit contains one Arc binding motif: aa 1384-1398. To confirm the interactions and pinpoint the amino acids critical for NMDA-Arc binding, a reconstitution system in human embryonic kidney (HEK) cells is used. Complex formation is established by co-transfection of Arc, NMDAR or their mutants into HEK-293T cells. Arc and putative binding proteins are co-immunoprecipitated (co-IP) and further detected with immunoblotting. Co-IP experiments have confirmed that NR2A and NR2B containing NMDARs form a complex with Arc in HEK cells. Co-expression of PDS-95 markedly enhances Arc-NMDAR complex formation. NR2A Arc binding motif deletion shows a decrease in NR2A pulldown. Ongoing co-IP experiments examine the effect of point mutations in NMDAR Arc-binding motifs that modulate NMDA-Arc binding. Additionally, since the NMDA receptor is a target of psychotomimic drugs, we will test NMDA receptor agonists, antagonists, and positive allosteric modulators in our reconstitution system to determine if they impair or strengthen the NMDA receptor-Arc-PSD-95 complex. This work will provide insights into the molecular mechanism of Arc -NMDAR binding, and a basis for understanding the role of this putative signaling complex in schizophrenia.

Disclosures: I.M. Gonzalez: None. **L. Yang:** None. **W. Zhang:** None. **P. Worley:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.11/B39

Topic: B.02. Ligand-Gated Ion Channels

Title: Defects in NMDA trafficking and stabilization associated with autism and intellectual disability-associated variants of the GluN2B subunit

Authors: *E. BAGATELAS, M. VIEIRA, K. ROCHE;
NINDS, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Excitatory receptors play an inherent role in proper brain function and are essential to neurodevelopment. Our study focuses on NMDARs (*N*-methyl-*D*-aspartate receptors), which mediate synaptic plasticity, a key mechanism underlying memory and learning. It has been illustrated that alterations to the NMDAR complex can have downstream effects on the synapses' capacity to function, and on downstream signaling processes. NMDARs form tetramers made up of different subunit combinations (GluN1; GluN2A-D; GluN3A-B), which allows fine-tuning of receptor function. Two of the most abundant of these subunits are GluN2B and GluN2A. These subunits have a striking developmental profile, with GluN2B subunits being highly expressed early in development and GluN2A levels increasing closer to birth. Dysfunction of these receptors yields improper formation of the nervous system, which could lead to the phenotypes observed in a variety of neurological and psychiatric disorders.
The GluN2B subunit is an essential component of NMDARs early in development, and previous studies have illustrated the critical importance of its C-terminus in stabilizing these receptors at the synapse. For example, the C-terminus of NMDARs is important for modulating receptor surface expression, intracellular trafficking, localization and downstream signaling. The GluN2B subunit has emerged in recent years as an important disease-causing gene in neurodevelopmental disorders, such as autism and intellectual disability (ID). Some of our previous research has shown phenotypes of autism-associated variants to have GluN2B receptor defects, in particular GluN2B S1413L (Liu et al., 2017.) We now focus on additional variants along the C-terminus identified in cases of neurodevelopmental disorders such as autism and ID, including missense and frameshift mutations. Our study looks at the phenotypic effects of these GluN2B variants using several different assays to assess their altered properties, including surface expression, trafficking and the study of the molecular complex associated with GluN2B-containing NMDARs. We have observed significant alterations in phenotypes, relative to WT (wild type) subunits in the parameters analyzed, demonstrating the impact of C-terminal variants on receptor trafficking and surface stability. By having a better understanding of what deficits occur at the molecular level of disease-causing genes, we hope to elucidate the receptor dysfunction underlying disease etiology.

Disclosures: E. Bagatelas: None. **M. Vieira:** None. **K. Roche:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.12/B40

Topic: B.02. Ligand-Gated Ion Channels

Title: Effects of patient-derived pathogenic anti-NMDA receptor antibodies on synaptic function and network activity

Authors: *E. ANDRZEJAK[1], F. ACKERMANN[1], C. ROSENMUND[2], N. ZIV[3], H. PRÜß[1], C. C. GARNER[1];
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Abstract: Over the last decade, a growing number of central nervous system (CNS) disorders have been linked to autoantibodies against synaptic and neuronal cell-surface proteins. These autoimmune encephalopathies present with a wide range of symptoms, from prominent psychiatric and cognitive manifestations, such as psychosis, distorted thoughts and memory loss, to severe seizures and autonomic instability. Studies of patient cerebro-spinal fluid (CSF) revealed that in a population of patients the antibodies recognize an epitope situated within the N-terminal domain of N-methyl-D-aspartate receptor (NMDAR), causing receptor cross-linking and subsequent internalization. However, these studies could not discriminate between the effects of anti-NMDAR and various other anti-neuronal antibodies present in patient CSF. Thus, we recently generated recombinant monoclonal antibodies cloned from single B cells from patients CSF, which give us a unique tool to characterize the specific contribution of anti-NMDAR antibodies to disease pathology. Using a combination of cell imaging assays and electrophysiological recordings, we investigated the effects of anti-NMDAR antibodies on single neuron function and network activity. We are currently examining the specificity of the antibody to various receptor and neuronal subpopulation. These data provide important insights into the specific mechanisms of anti-NMDAR encephalitis and possibly explain its diverse symptomatology.

Disclosures: E. Andrzejak: None. **F. Ackermann:** None. **C. Rosenmund:** None. **H. Prüß:** None. **C.C. Garner:** None. **N. Ziv:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.13/B41

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH/NIMH Grant F30MH115618
NIH/NINDS Grant NS088479
NIH/NIA/NIAID Grant 5P01AI073693-10

Title: Lupus autoantibodies selectively target GluN2A-containing NMDA receptors to induce chronic spatial memory defects

Authors: *K. CHAN[1], J. NESTOR[3], T. S. HUERTA[3], G. MOODY[2], N. CERTAIN[2], C. KOWAL[3], P. T. HUERTA[3], B. T. VOLPE[3], B. DIAMOND[3], L. P. WOLLMUTH[1]; [1]Neurobiol & Behavior, [2]Pharmacol., Stony Brook Univ., Stony Brook, NY; [3]Feinstein Inst. For Med. Res., Manhasset, NY

Abstract: Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease where patients may develop perturbations in neurological and psychiatric function. A subset of these patients produce anti-dsDNA antibodies (DNRAbs) that cross-react with the NMDA receptor. DNRAbs mediate acute synaptic dysfunction and excitotoxicity largely in the hippocampus, later manifesting into impaired spatial memory and place field defects. NMDA receptors are ionotropic glutamate receptors, obligate heterotetramers that are typically composed of two GluN1 subunits and two GluN2 subunits. The major GluN2 subunits in the CA1 region of the hippocampus are GluN2A and GluN2B. These subunits have vastly different pharmacological and physiological properties, yet how DNRAbs specifically exert their cytotoxic effects through each NMDA receptor subtype is largely unknown. Here, we use a combination of ion channel biophysics and lupus mouse models to interrogate the subtype specificity of DNRAbs on NMDA receptors. We find that DNRAbs act as positive allosteric modulators (PAMs) on NMDA receptors with GluN2A-containing NMDA receptors exhibiting much greater sensitivity to DNRAbs than those with exclusively GluN2B at the whole-cell and single-channel level. Accordingly, GluN2A-specific antagonists provide greater protection from DNRAb-mediated neuronal cell death than GluN2B antagonists. Using transgenic mice to perturb expression of either GluN2A or GluN2B *in vivo* that are immunogenized to generate lupus autoantibodies, we find that DNRAb-mediated disruption of spatial memory characterized by early neuronal cell death and subsequently microglia-dependent synaptic pruning in the hippocampus requires GluN2A, but not GluN2B. Our results suggest that GluN2A-specific antagonists or negative allosteric modulators are strong candidates to treat SLE patients with neuropsychiatric dysfunction.

Disclosures: K. Chan: None. **J. Nestor:** None. **T.S. Huerta:** None. **G. Moody:** None. **N. Certain:** None. **C. Kowal:** None. **P.T. Huerta:** None. **B.T. Volpe:** None. **B. Diamond:** None. **L.P. Wollmuth:** None.

Poster

034. Ionotropic Glutamate Receptors: Physiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 034.14/B42

Topic: B.02. Ligand-Gated Ion Channels

Support: Deutsche Forschungsgemeinschaft funded Graduate College 1657

Title: Role of physiological breaks and ionizing radiation in expression of immediate early genes

Authors: R. KAUR, ***B. LAUBE**;
TU Darmstadt, Darmstadt, Germany

Abstract: Immediate early genes (IEGs) contribute to neuronal plasticity and memory formation. It has been shown that neuronal activity and subsequent NMDAR-stimulation by glutamate triggers the formation of type IIß topoisomerase (Top2ß)-mediated double strand breaks (DSBs) in the promoters of IEGs in neurons. These activity-induced Top2β-mediated DSBs are thought to be crucial for the experience-driven changes in neuronal function associated with learning and memory. However, DSBs are arguably the most deleterious DNA lesion and are induced upon irradiation (IR). Several data suggest that IR also modifies gene expression, which is considered to be a critical component of IR-induced brain damage and neuronal radiosensitivity. Thus, the interference of NMDAR- and IR-dependent DNA damage and repair could be a mechanism having important physiological and pathological implications for neuronal function by regulating gene expression. Therefore we intended to analyze in a comparative study the expression profile of distinct genes upon NMDAR- and IR-induced DSBs to get insights in the mechanisms and the signal pathways involved in altering expression of IEGs. The first aim was to examine the impact of NMDA and IR on i) Top2β DSB-induction and ii) physiological and cellular functions in neurons to see whether IR-induced DSBs interfere with the function of activity-induced DNA DSBs. We found in primary hippocampal neurons cultivated for 14 days that NMDA incubation increased DSBs by staining against the phosphorylated form of histone variant H2AX whereas application of NMDAR blockers prevented DSB induction. NMDAR-mediated DSBs diminished within 2 hours after application indicating that these DSBs are transient and repaired by NHEJ. Reverse transcription polymerase chain reaction (RT-PCR) and Western Blot data showed an increase in relative fold expression of IEGs when neurons were treated with NMDA, the Top2ß-inhibitor Etoposide, or in combination and revealed a decrease upon NMDAR antagonist application. Functional analysis of the cultures by Multielectrode array (MEA) chips showed an increase in spike activity upon NMDA and Etoposide treatment, which indicates a correlation of IEG expression and neuronal function in our cultures. Preliminary results upon IR showed that lower doses (<1 Gy) lead to a decrease in both, IEGs expression and spike activity, whereas higher doses had an opposite effect. In conclusion, our data provide new insights in the impact of activity- and IR-dependent DSBs in the regulation of IEG expression in neurons.

Disclosures: R. Kaur: None. **B. Laube:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.01/B43

Topic: B.04. Ion Channels

Support: UCB Neuroscience Grant
Australian Brain Foundation
Royal Melbourne Hospital Neuroscience Foundation Grant

Title: A novel formalism to replace the Hodgkin Huxley model for sodium current simulations

Authors: *C. FRENCH, J. N. WINDERLICH;
Univ. of Melbourne, Melbourne, Australia

Abstract: Introduction Most neuronal modelling relies on the over 60 year old Hodgkin Huxley equations, but these are based on squid axon currents and are known to be deficient in certain aspects. They use voltage-dependent open state inactivation, which is known to be incorrect, do not display biexponential open state inactivation, do not produce a significant persistent sodium current (I_{Nap}) and have activation kinetics inconsistent with those observed in central neurons in recent high-resolution recordings
We present a new model that is simpler in structure than H-H and replicates all the above properties by implementing a novel state topology which allows opening from all closed states into two open states and a single fast inactivated state.
Methods and Results Voltage clamped sodium currents were recorded from acutely dissociated rat CA1 hippocampal neurons as well as human Nav 1.2 heterologously expressed HEK cells at ~12C. These currents were then fitted to a 5 state Markov model using a "swarm" algorithm to optimise parameters.
This model was able to fit graded exponentiated activation kinetics, biexponential inactivation and INap properties very accurately. Additionally, the open state inactivation was non-voltage sensitive as found in gating current experiments. Subsequent modelling at the single cell network level revealed reduced time to firing for single neurons and different resonant frequency behaviour of conductance based cortical microcircuit models. The model has been implemented in Matlab and Neuron and can be easily substituted into models using these platforms.
Conclusions A much more accurate model of central neuron sodium currents with reduced complexity has been developed that can be substituted for the HH equations.

Disclosures: C. French: None. **J.N. Winderlich:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.02/B44

Topic: B.04. Ion Channels

Support: 1R01MH111107
R01 MH095995

T32 AG051131
T32 GM089657
Jeane B. Kempner Postdoctoral Fellowship

Title: Identification and functional validation of allosteric modulators of Nav 1.1 channels

Authors: *A. K. SINGH[1], J. WONG[1], S. ALI[1], P. WADSWORTH[1,2], O. FOLORUNSO[1], F. LAEZZA[1];
[1]Pharmacol. & Toxicology, The Univ. of Texas Med. Br. (UTMB), Galveston, TX; [2]Biochem. & Mol. Biol., Combined MD/PhD Program, The University of Texas Medical Branch (UTMB), TX

Abstract: Voltage-gated sodium (Nav) channels provide the basis for neuronal excitability in the brain. Of the nine Nav channel isoforms (Nav1.1-Nav1.9), Nav1.1 exhibits cell-specific distribution in fast-spiking parvalbumin (PV) interneurons in the cortical circuit. Reduced firing of these cells is a common feature in neuropsychiatric and neurodegenerative disorders, raising the need to develop selective allosteric modulators targeting Nav1.1. The fibroblast growth factor 14 (FGF14) directly binds to the C-terminal tail of Nav channels resulting in isoform-specific modulation of Na+ currents and channel biophysical properties. These unique structure-function properties of protein:protein interaction (PPI) interfaces between FGF14 and different Nav isoforms could provide a novel target for developing isoform-specific small molecule modulators of Nav channels. Here, we have conducted a ligand-based high-throughput virtual screening against the FGF14:Nav1.1 complex using Autodock. We used a grid box encompassing a portion of the FENYYV sequence (residues 155-160) of FGF14 within a distance of 8Å from the Nav1.1 C-tail; this region is part of a previously identified druggable pocket within the β9 sheet of FGF14. We initially identified 1001 ZINC compounds predicted to bind this interaction site out of 642,759 screened ligands, and these were further narrowed down to 14 hits based on putative binding scores. Finally, we selected ZINC1 and ZINC3 for further studies based on chemical properties, including predicted cLogP. Surface plasmon resonance and whole-cell patch clamp electrophysiology confirmed binding of ZINC3 to FGF14 and functional activity of the compound against Nav1.1-mediated Na+ currents. Interestingly, ZINC3 functional effect on Nav1.1 currents was FGF14 isoform-dependent. The compound suppressed Nav1.1 peak transient currents ($n=14$, $p<0.0031$) and decreased channel availability through regulation of long-term inactivation ($n=14$, $p<0.005$) in the presence of FGF14-1b, while induced a depolarizing shift in the voltage-dependence of activation in the presence of FGF14-1a ($n=6$, $p<0.002$). In conclusion, ZINC3 and other small molecules targeting the FGF14:Nav channel PPI interface could serve as scaffolds to develop Nav channel isoform-specific allosteric modulators with broad applicability for neuropsychiatric and neurodegenerative disorders.

Disclosures: A.K. Singh: None. **J. Wong:** None. **S. Ali:** None. **P. Wadsworth:** None. **O. Folorunso:** None. **F. Laezza:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.03/B45

Topic: B.04. Ion Channels

Title: Use of reference human induced pluripotent stem cell (iPSC) models to study SCN2A variants associated with epilepsy and autism spectrum disorders

Authors: *Z. QUE[1], M. OLIVERO-ACOSTA[1], J. ZHANG[1], Y. LIU[1], W. SKARNES[2], Y. YANG[1];
[1]Purdue Univ., West Lafayette, IN; [2]The Jackson Lab., Farmington, CT

Abstract: Nav1.2, a voltage-gated sodium channel encoded by *SCN2A*, is responsible for action potential firing and propagation in the central nervous system. Due to the wide adoption of whole gene sequencing over the past few years, a strong correlation has been established between genetic variants of *SCN2A* and neurological diseases including epilepsy, autism spectrum disorders (ASDs), intellectual disability among others. Gain-of-function variants are suggested to increase excitability of neurons which may lead to epilepsy, whereas loss-of-function variants are considered to be associated with ASDs. To understand how some of the major recurring variants of *SCN2A* perturb neurons and alter neuronal excitability, we used CRIPSR/Cas9 to create *SCN2A* disease-associated variants in a human reference iPSC line (KOLF2-C1). Using reference iPSCs enables these variants to be engineered rapidly and assessed within the same genetic background, eliminating the potentially confounding contribution of other genetic alleles present in individual patients. We are using patch-clamping to study biophysical properties and excitability of neurons derived from these engineered iPSCs. The data we present would validate the feasibility of using iPSC disease models to study the corresponding phenotypes and to elucidate disease mechanisms caused by *SCN2A* variants identified from patients with these neurological diseases.

Disclosures: Z. Que: None. **M. Olivero-Acosta:** None. **J. Zhang:** None. **Y. liu:** None. **W. Skarnes:** None. **Y. yang:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.04/B46

Topic: B.04. Ion Channels

Support: NSF GRFP DGE-1842166
Ralph W. and Grace M. Showalter Research Trust

Title: Neuronal properties and neural network excitabilities in a mouse model with substantial reduction of Scn2a expression

Authors: *J. ZHANG, M. EATON, Z. QUE, Z. MA, C. ZHANG, A. PARK, C. ROMERO, Y. YANG;
MCMP, Purdue Univ., West Lafayette, IN

Abstract: Voltage-gated sodium channels including Nav1.2 (encoded by gene *SCN2A*) play essential roles in initiating action potentials in central nervous system. Loss-of-function variants of Nav1.2 are closely related to autism spectrum disorder (ASD), intellectual disability and other neurodevelopmental disorders. However, the relationship between dysfunction of Nav1.2 channel and neuronal excitabilities underlying pathophysiological conditions remains elusive. We acquired a mouse model in which *Scn2a* is expressed in a substantially low level without perinatal lethality. Using whole-cell patch-clamp recording in brain slices, we are performing a study to understand how neuronal properties and neural network excitabilities are altered when *Scn2a* expression level is profoundly reduced. Additionally, morphologic changes of principal neuronal dendritic trees and spines in different brain regions are being explored in this mouse model. The data we obtain will elucidate how Nav1.2 channel affects neuronal properties and how the reduction of *Scn2a* expression leads to pathophysiological conditions, laying a foundation for further understanding of Nav1.2-related neurodevelopmental disorders.

Disclosures: J. Zhang: None. **M. Eaton:** None. **Z. Que:** None. **Z. Ma:** None. **C. Zhang:** None. **A. Park:** None. **C. Romero:** None. **Y. Yang:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.05/B47

Topic: B.04. Ion Channels

Support: NSF GRFP
Ralph W. and Grace M. Showalter Research Trust

Title: Molecular characterization and seizure susceptibility analysis of a mouse model with substantial reduction in Scn2a expression levels

Authors: *Z. MA[1,2], **A. PARK**[1], M. EATON[1], J. ZHANG[1], C. ZHANG[1], E. LIETZKE[1], N. LANMAN[3], W. SKARNES[4], Z. HUANG[2], Y. YANG[1];
[1]Dept. of Medicinal Chem. and Mol. Pharmac, West Lafayette, IN; [2]Dept. of Mol. and Cell. Pharmacol., Peking Univ. Hlth. Sci. Ctr., Beijing, China; [3]Dept. of Comparative Pathobiology, West Lafayette, IN; [4]The Jackson Lab. for Genomic Med., Farmington, CT

Abstract: Dysfunctions of sodium channel Nav1.2, encoded by the *Scn2a* gene, is closely associated with autism spectrum disorder (ASD) and epilepsy. Nav1.2 is crucial for the generation and backpropagation of the action potential (AP) in central nervous system. Canonical methods of disrupting *Scn2a* coding exons produced homozygous mice (*Scn2a*$^{-/-}$) that die a few days after birth. Heterozygous mice (*Scn2a*$^{+/-}$), on the other hand, display mild abnormalities but do not recapitulate severe disease phenotypes seen in patient. Here we report a mouse model in which the expression of *Scn2a* is substantially, but not completely, reduced. These mice can survive to adulthood, likely due to the residual Nav1.2. A profound reduction in *Scn2a* expression levels was confirmed by whole brain qPCR, RNAseq, and Western blot analysis. Bioinformatic analysis of the RNAseq data is being performed to understand how substantial reduction of *Scn2a* expression may affect the molecular landscape of neurons in different brain regions. The expression pattern of *Scn2a* in the mouse brain is also being studied. Since dysfunction of *Scn2a* is associated with epilepsy and classic *Scn2a*$^{+/-}$ heterozygous mice were suggested to have absence-like seizure, we are currently studying the seizure susceptibility of our mouse model using electroencephalogram (EEG) as well other technologies. Our studies will contribute to the understanding of *Scn2a* in a variety of neurological diseases, especially the diseases caused by loss-of-function of *Scn2a* including ASD and its seizure comorbidities.

Disclosures: Z. Ma: None. **A. Park:** None. **M. Eaton:** None. **J. Zhang:** None. **C. Zhang:** None. **E. Lietzke:** None. **N. Lanman:** None. **W. Skarnes:** None. **Z. Huang:** None. **Y. Yang:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.06/B48

Topic: B.04. Ion Channels

Support: NSF GRFP
Ralph W. and Grace M. Showalter Research Trust

Title: Substantial reduction of Scn2a expression renders behavioral abnormalities in mice indicative of autism spectrum disorder

Authors: *M. EATON, J. ZHANG, Z. MA, C. ZHANG, A. PARK, E. LIETZKE, Y. YANG; Medicinal Chem. and Mol. Pharmacol., Purdue Univ., West Lafayette, IN

Abstract: Recent whole exome sequencing studies have discovered a strong correlation between the voltage-gated sodium channel Nav1.2 (encoded by gene *SCN2A*) and autism spectrum disorder (ASD), as well as other neurodevelopmental disorders. Nav1.2, together with Nav1.1 and Nav1.6 which are major sodium channels also expressed in the central nervous system, regulates neuronal excitability. Homozygous knockout of *Scn2a* $^{-/-}$ results in perinatal lethality in mice. Heterozygous *Scn2a* $^{+/-}$ mice display mild abnormalities in some assays but do not seem to recapitulate severe disease phenotypes. To further explore the relationship between the dysfunction of Scn2a and ASD and its comorbidities, our lab acquired a mouse model in which the expression of *Scn2a* is substantially reduced. We are currently conducting a behavioral battery to assess the phenotypes of these mice based on the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) definition of ASD as well as assessing whether other reported comorbidities of ASD (i.e. learning and memory deficits, anxiety) are present. We plan to test both males and females to determine if there are sex differences. The data we obtain will justify that these mice can be used as a model to study severe ASD and its related comorbidities associated with *SCN2A* loss-of-function mutations.

Disclosures: **M. Eaton:** A. Employment/Salary (full or part-time):; Purdue University. **J. Zhang:** A. Employment/Salary (full or part-time):; Purdue University. **Z. Ma:** A. Employment/Salary (full or part-time):; Purdue University. **C. Zhang:** A. Employment/Salary (full or part-time):; Purdue University. **A. Park:** None. **E. Lietzke:** None. **Y. Yang:** A. Employment/Salary (full or part-time):; Purdue University.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.07/B49

Topic: B.04. Ion Channels

Support: NIH/NINDS Grant U54NS108874
NIH/NINDS Grant R01NS053422

Title: Palmitoylation distinctively modulates Nav1.6 and Nav1.2

Authors: *Y. PAN[1], T. R. CUMMINS[2];
[1]Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Dept Biol. SL306, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Palmitoylation is a reversible post-translational lipid modification that dynamically regulates protein trafficking and membrane association. Voltage-gated sodium (Nav) channels are subjected to palmitoylation and exhibit altered functional properties in different palmitoylation states. Our aim was to investigate whether and how palmitoylation regulates Nav1.6 channel functions, and to identify palmitoylation sites in Nav1.6 that can potentially be pharmacologically targeted. Using the acyl-biotin exchange assay, we found that Nav1.6 is modified by palmitoylation in the mouse brain as well as in the HEK cell line stably expressing Nav1.6. With whole-cell voltage clamp, we discovered that disrupting palmitate incorporation with 2-Br-palmitate resulted in a 67% reduction of Nav1.6 current and a 8mV hyperpolarizing shift of the voltage-dependence of inactivation. Enhancing palmitoylation with palmitate acid, the substrate for palmitoylation, increased Nav1.6 current by 78%. To identify the palmitoylation sites responsible for these functional alterations, we substituted three cysteines (C1169, C1170, C1978), predicted to be palmitoylated in Nav1.6, with alanines. We found that the double cysteine mutant (C1169,1170A) replicated the hyperpolarizing shift observed with 2-Br-palmitate treatment, while C1978 was responsible for the current density effect. Interestingly, C1978 is exclusive to Nav1.6 among all isoforms of voltage-gated sodium channels and it is evolutionally conserved among most species. This suggests an important role of C1978 palmitoylation in regulating Nav1.6 functions. Indeed, Nav1.2 (without the homologous C1978) did not exhibit increased current density when treated with palmitate acid. However, when a cysteine was introduced to the homologous site in Nav1.2 (K2005C), the mutant channel displayed similar current density response to palmitate acid treatment as Nav1.6, affirming the unique role of Nav1.6 C1978 in regulating channel current density. Therefore, palmitoylation may serve as a novel isoform-specific mechanism to modulate neuronal excitability in physiological and diseased conditions.

Disclosures: Y. Pan: None. **T.R. Cummins:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.08/B50

Topic: B.04. Ion Channels

Title: CaMKII modulates hNav1.6 sodium currents at multiple phosphorylation sites

Authors: *A. ZYBURA[1], A. J. BAUCUM II[2], T. R. CUMMINS[3], A. HUDMON[4];
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Abstract: Sodium currents produced by Nav1.6 are critical for action potential initiation and propagation in neurons. Aberrant alterations to Nav1.6 activity and expression are linked to disorders of excitability, including epilepsy and pain. Reversible phosphorylation of Nav alpha subunits by kinases is a powerful mechanism that can mediate these changes and consequently modify neuronal function in synaptic physiology and pathology. The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a kinase that is critical for neuronal excitability and cellular plasticity. Although this kinase is known to modulate Nav isoforms critical for cardiac and neuronal function, the ability of CaMKII to modulate Nav1.6, the primary voltage-gated sodium channel in mature axon initial segments and nodes, is unknown. In this study we use nanoflow liquid chromatography coupled with electrospray-ionization mass spectrometry (LC/ESI-MS) on Nav1.6 affinity-purified from HEK293 cells stably expressing the channel under conditions facilitating CaMKII activation or inhibition to show that phosphorylation is predominantly limited to the first intracellular loop (L1) of Nav1.6. Here we identify 11 phosphorylation sites located in this region, 3 of which are novel CaMKII phosphorylation sites (S561, S641 and T642) and the remainder phosphorylated by other kinases. Functional analysis shows that inhibition of CaMKII with CN21 in neuronal ND7/23 cells transiently expressing TTX-resistant Nav1.6 results in a significant reduction in Nav1.6 transient and persistent current density, with negligible effects on voltage-dependent gating properties. We show that the substitution of S561, S641 or T642 with an aspartic acid mimics phosphorylation and prevents CaMKII inhibition-mediated reductions in sodium currents. Alanine substitution at S641 and T642 ablates these effects. Our study is the first to demonstrate CaMKII phosphorylation and modulation of the critical neuronal voltage-gated sodium channel Nav1.6 and may suggest a novel mechanism in regulating neuronal excitability.

Disclosures: **A. Zybura:** None. **A.J. Baucum II:** None. **T.R. Cummins:** None. **A. Hudmon:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.09/B51

Topic: B.04. Ion Channels

Support: NIH Grant NS34509

Title: Anti-sense oligonucleotide therapy delays seizure onset and extends survival in a mouse model of SCN8A encephalopathy

Authors: G. M. LENK[1], P. JAFAR-NEJAD[4], L. D. HUFFMAN[2], C. SMOLEN[1], J. L. WAGNON[1], H. PETIT[1], R. J. GIGER[3], F. RIGO[4], ***M. H. MEISLER**[1];

[1]Human Genet., [2]Neurosci., [3]Neurology/Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI; [4]Ionis Pharmaceuticals, Carlsbad, CA

Abstract: *SCN8A* encephalopathy results from *de novo* gain-of-function mutations affecting the sodium channel $Na_v1.6$ that lead to neuronal hyperactivity. Affected individuals exhibit early onset seizures, developmental delay, and cognitive impairment, and are not well controlled by available therapies. We evaluated the effectiveness of anti-sense oligonucleotide (ASO) treatment using a conditional mouse model with Cre-dependent expression of the patient mutation p.Arg1872Trp. Mice were treated with an ASO that decreased the abundance of the *Scn8a* transcript by 40% after intracerebroventricular injection of neonatal mice. A dose-dependent increase in survival from 2 weeks to 7 weeks was observed, with a further increase to 9 weeks after a second dose of ASO. The treated mice did not exhibit the movement disorders seen in spontaneous hypomorphic mutants that have 90% reduction of transcript, such as ataxic gait, dystonia, hind limb paralysis or muscle wasting. Sciatic nerve conduction velocity was reduced by 25% in ASO treated mice without a visible effect on mobility. The data demonstrate the potential utility of ASO therapy for this intractable childhood epilepsy.

Disclosures: G.M. Lenk: None. **P. Jafar-Nejad:** None. **L.D. Huffman:** None. **C. Smolen:** None. **J.L. Wagnon:** None. **H. Petit:** None. **R.J. Giger:** None. **F. Rigo:** None. **M.H. Meisler:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.10/B52

Topic: B.04. Ion Channels

Support: PhRMA Foundation Fellowship in Pharmacology
NIH/NIA Grant 5T32AG051131
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NIH/NIMH Grant R01MH095995
John Sealy Memorial Endowment Funds
UTMB MD/PhD Combined Degree Program

Title: Discovery of FGF14:Nav1.6 complex modulators by high-throughput screening against protein:protein interactions

Authors: *P. A. WADSWORTH[1], A. K. SINGH[1], O. FOLORUNSO[2], N. D. NGUYEN[3], D. BRUNELL[3], C. C. STEPHAN[3], F. LAEZZA[1];
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Abstract: Ion channel macromolecular complexes play a critical role in regulating and finely tuning neuronal firing. Minimal disturbances to these tightly controlled protein:protein interactions (PPI) can lead to persistent maladaptive plasticity of brain circuitry. However, these PPI interfaces are highly specific and provide ideal targets for drug development, especially in the CNS where selectivity and specificity are vital for limiting side effects. We present the initial results of a high-throughput drug screening (HTS) campaign targeting the PPI interface of the voltage-gated Na^+ (Nav) channel 1.6 and its regulatory protein, fibroblast growth factor 14 (FGF14). The FGF14:Nav1.6 complex is enriched in medium spiny neurons in the nucleus accumbens, and therefore compounds targeting this complex could bring about a new class of anti-depressants or mood stabilizers. Following assay optimization in 384-well plates, we conducted an in-cell HTS against the FGF14:Nav1.6 complex using the split-luciferase complementation assay (LCA). We screened ~50,000 small molecules and rationally-designed drug-like analogues in duplicate, and compound Z-scores were calculated by normalizing luminescence to per plate controls (0.3% DMSO). A fluorescence-based cell viability assay was conducted in parallel, and potentially toxic compounds were excluded ($Z \leq -3$). Using cut-offs of $Z \leq -5$ for inhibitors and $Z \geq 3$ for enhancers, we initially identified 960 hits. Of these, 640 compounds failed to achieve significance during validation screening (n=3), and an additional 149 were identified as false positives based on counter-screening against luciferase ($Z \leq -3$ or $Z \geq 3$). The remaining 168 hits were then stratified by structural and chemical properties including predicted permeability (logP), and an initial dose response was conducted for 60 compounds with the greatest potential for blood-brain barrier permeability. We repurchased 26 promising compounds for validation by an expanded 10-point dose response, and hits were then ranked based upon their potency (EC_{50}/IC_{50}) and efficacy. Estimated in-cell IC_{50} of the top 14 inhibitors ranged from 0.95 to 15 µM, while estimated EC_{50} of the top 4 enhancers ranged from 0.65 to 1.21 µM. Cell-free orthogonal screenings including surface plasmon resonance (SPR), protein thermal shift (PTC), and isothermal titration calorimetry (ITC) were subsequently used to assess hit binding affinity for purified FGF14 and Nav1.6 protein, and *in silico* docking was used to predict potential binding sites. Promising hits are now being functionally evaluated as modulators of Nav1.6 currents and neuronal firing from MSNs in the nucleus accumbens.

Disclosures: P.A. Wadsworth: None. **A.K. Singh:** None. **O. Folorunso:** None. **N.D. Nguyen:** None. **D. Brunell:** None. **C.C. Stephan:** None. **F. Laezza:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.11/B53

Topic: B.04. Ion Channels

Title: Selective pharmacological inhibition of sodium channel isoforms $Na_v1.6$ and $Na_v1.2/1.6$ attenuates action potential firing in mouse cortical pyramidal neurons while sparing firing in inhibitory interneurons

Authors: *A. D. WILLIAMS, S. J. GOODCHILD, N. SHUART, K. KHAKH, W. GONG, A. HASAN, T. FOCKEN, C. COHEN, J. EMPFIELD, J. P. JOHNSON;
Xenon Pharmaceuticals, Burnaby, BC, Canada

Abstract: Despite many years of effort towards drug development, about 30% of patients with newly diagnosed epilepsy eventually become refractory to treatment (French, 2007; Regesta and Tanganelli, 1999). A clear need exists for the development of novel anti-epileptic therapies. Most commonly prescribed anti-epileptic drugs (AEDs) have different therapeutic profiles and efficacy on multiple molecular targets (Rogawski et al., 2015; Remy and Beck, 2006). Many of these drugs are non-selective inhibitors of voltage-gated sodium channels. However, sodium channel isoform distribution is distinct and non-uniform within neurons in the brain. In the CNS of wild-type mice at P21 and beyond, the $Na_v1.1$ isoform is believed to dominate in the axon initial segment (AIS) of inhibitory interneurons (Ogiwara et al., 2007; Yu et al., 2006). In contrast, principal neuron sodium channel expression is believed to be dominated by $Na_v1.2$ and $Na_v1.6$, with $Na_v1.6$ expression dominant in the nodes of Ranvier and AIS after about P20, while $Na_v1.2$ expression dominates in the AIS at P15 and earlier (Liao et al., 2010; Hu et al., 2009; Caldwell et al., 2000). Therefore, a non-selective sodium channel inhibitor might impair action potential (AP) firing in both excitatory and inhibitory neurons, diminishing the desired effect of reduced network hyperexcitability. Xenon has developed highly selective inhibitors of the $Na_v1.6$ and $Na_v1.2/1.6$ isoforms, and here describe their effect on AP firing in pyramidal neurons and interneurons in mouse brain slices. Selective inhibition of $Na_v1.6$ with bath application of 500 nM XPC-7224 (~3X IC50 for $Na_v1.6$ stably expressed in HEK293 cells) significantly inhibits firing of pyramidal neurons in cortical layer 2/3 pyramidal neurons in brain slices from adult CF-1 mice, as measured by current clamp electrophysiology. However, the same concentration of XPC-7224 had markedly less impact on AP firing in cortical inhibitory interneurons. Similarly, inhibition of $Na_v1.2/1.6$ with bath application of 150 nM XPC-5462 (~3X IC50) impairs AP firing in cortical pyramidal neurons with only modest effect on AP firing in cortical inhibitory interneurons. In contrast, bath application of 100 μM carbamazepine (~3X IC50), a commonly prescribed AED, impairs AP firing in both pyramidal neurons and interneurons in brain slices from age-matched mice. $Na_v1.6$ and $Na_v1.2/1.6$ selective molecules attenuated AP firing in excitatory pyramidal neurons, while sparing the inhibitory function of interneurons. We anticipate that this profile could lead to improved efficacy in epilepsy patients relative to currently marketed non-selective sodium channel-inhibiting AEDs.

Disclosures: **A.D. Williams:** A. Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **S.J. Goodchild:** A. Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **N. Shuart:** A. Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **K. Khakh:** A. Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **W. Gong:** A. Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **A. Hasan:** A.

Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **T. Focken:** A.
Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **C. Cohen:** A.
Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **J. Empfield:** A.
Employment/Salary (full or part-time):; Xenon Pharmaceuticals. **J.P. Johnson:** A.
Employment/Salary (full or part-time):; Xenon Pharmaceuticals.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.12/B54

Topic: B.04. Ion Channels

Support: EU Horizon 2020 research and innovation programme Marie Sklodowska-Curie grant for PAIN-Net, Molecule-to-man pain network (grant no. 721841)
US Dept Veterans Affairs B9253-C

Title: Pharmacogenomic analysis of effect of lacosamide on hNav1.7 variants from responsive and non-responsive small fiber neuropathy patients

Authors: *M. R. ESTACION[1,2], **J. LABAU**[1], B. TANAKA[1], B. T. A. DE GREEF[3,4], J. G. J. HOEIJMAKERS[3], M. GEERTS[3], M. GERRITS[5], H. J. M. SMEETS[6], C. G. FABER[3], I. S. J. MERKIES[3,7], S. G. WAXMAN[1,2], S. D. DIBB-HAJJ[1,2];
[1]Yale Univ. Sch. of Med., New Haven, CT; [2]DVA, West Haven, CT; [3]Dept. of Neurology, Sch. of Mental Hlth. and Neurosci., [4]Dept. of Clin. Epidemiology and Med. Technol. Assesment (KEMTA), [5]Dept of Clin. Genet., [6]Dept of Genet. and Cell Biology, Clin. Genomics Unit, Maastricht Univ., Maastricht, Netherlands; [7]Dept of Neurol., St. Elisabeth Hosp., Willemstad, Curaçao

Abstract: *Background*: A direct role of sodium channels in pain has recently been confirmed by establishing a monogenic link between SCN9A, the gene which encodes sodium channel Nav1.7, and pain disorders in humans, with gain-of-function mutations causing severe pain syndromes, and loss-of-function mutations causing congenital indifference to pain. Lacosamide has been clinically approved as an anti-epileptic drug and targets voltage-gated sodium channels. The mechanism of action of Lacosamide shows slow kinetics, a finding that has been interpreted as suggesting a preferential effect on the slow-inactivated state compared to other compounds that are thought to bind to the local anesthetic site. Here we used voltage-clamp recordings to evaluate the effects of Lacosamide on Nav1.7 variants carried by patients (responders and non-responders) that participated in a trial on the clinical effectiveness of Lacosamide for relieving pain against small fiber neuropathy.
Results: At the clinically achievable concentration of 30uM, we characterized the effect of Lacosamide on use-dependent block, fast-inactivation and slow-inactivation. We observed

different effects of Lacosamide in these protocols when comparing different hNav1.7 variants. *Conclusions*: Comparison of the clinical responses to Lacosamide to the hNav1.7 variant that the patient carried suggests that for certain variants, clinical responsiveness could be correlated with in-vitro drug responses.

Disclosures: M.R. Estacion: None. **J. Labau:** None. **B. Tanaka:** None. **B.T.A. de Greef:** None. **J.G.J. Hoeijmakers:** None. **M. Geerts:** None. **M. Gerrits:** None. **H.J.M. Smeets:** None. **C.G. Faber:** None. **I.S.J. Merkies:** None. **S.G. Waxman:** None. **S.D. Dibb-Hajj:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.13/B55

Topic: B.04. Ion Channels

Support: Medical Research Service and Rehabilitation Research Service
Department of Veterans Affairs

Title: Nav1.8 gain-of-function mutation in a patient with familial trigeminal neuralgia

Authors: *J.-H. YUAN[1], G. DI STEFANO[2], F. DIB-HAJJ[1], G. CRUCCU[2], S. G. WAXMAN[1], A. TRUINI[2], S. D. DIB-HAJJ[1];
[1]Neurol., Yale Sch. of Med., New Haven, CT; [2]Human Neurosciences, Sapienza Univ., Rome, Italy

Abstract: Background Trigeminal neuralgia (TN) is chronic pain disorder characterized by paroxysmal pain in one or more divisions of the trigeminal nerve, typically evoked by innocuous stimuli. Severe neurovascular compression (NVC) of the nerve has been linked to classical TN, but mild or moderate NVC is not sufficient to cause TN, suggesting contribution by additional genetic and/or epigenetic factors. Approximately 2% of TN patients have a positive family history, while the aim of this study is to investigate potential genetic components that contribute to familial TN.
Methods We collected DNA sample from a 73-year-old man with a family history of TN; his deceased father had also suffered from a similar disorder. The subject started to experience paroxysmal pain of the first trigeminal division at age 63, and subsequently developed concomitant continuous pain. MRI identified a moderate dislocation of trigeminal root with two conflicting vessels. Whole-exome sequencing (WES) was carried out using Illumina NovaSeq 6000. Rodent/human RNA sequencing data of trigeminal ganglia were applied for evaluation of the expression of candidate genes.
Results Among 503 uncommon genomic variants, we found 10 genes registered on the Human Pain Genetics Database. Missense variants were identified in four genes related to electrogenesis

of sensory neurons, comprising voltage-gated sodium channel genes *SCN9A* (encodes Nav1.7) and *SCN10A* (Nav1.8), chloride intracellular channel (CLIC5), and potassium voltage-gated channel (KCNJ6). Therein, the heterozygous p.Ala1304Thr mutation in Nav1.8 was previously described in a female patient with episodic stabbing pain in distal extremities, and was shown to increase excitability of sensory neurons. Sequencing analysis of two unaffected siblings (a brother and a sister) validated that only the Nav1.8-A1304T mutation was unique to the affected brother.
Conclusions We identified a gain-of-function mutation in *SCN10A* in a patient with classical TN and a positive family history. The previously characterized Nav1.8-A1304T mutation has been shown to increase excitability of sensory neurons and thus can be considered as likely to contribute to TN in the affected subject. To our knowledge, *SCN10A* has never been linked to the TN phenotype, and our findings highlight the crucial role of sodium channels in the pathophysiology underlying TN.

Disclosures: J. Yuan: None. **G. Di Stefano:** None. **F. Dib-Hajj:** None. **G. Cruccu:** None. **S.G. Waxman:** None. **A. Truini:** None. **S.D. Dib-Hajj:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.14/B56

Topic: B.04. Ion Channels

Support: Grants from the Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

Title: A 49 amino acid stretch governs functional expression of sodium channel 1.9 (Nav1.9) in HEK-293 cells

Authors: *D. V. SIZOVA[1,2], J. HUANG[1,2], E. J. AKIN[1,2], S. G. WAXMAN[1,2], S. D. DIB-HAJJ[1,2];
[1]Neurol., Yale Univ. Sch. of Med., New Haven, CT; [2]Ctr. for Neurosci. and Regeneration Res., West Haven, CT

Abstract: Nav1.9 has been shown to play an important role in human pain. Low functional expression in heterologous systems has hampered progress for a better understanding of Nav1.9 electrophysiological and pharmacological properties. The purpose of this study was to better understand molecular mechanisms that govern functional expression of Nav1.9 and to identify its molecular determinants.
First, we explored the ability of Nav1.9 to be inserted within the plasma membrane in transiently transfected HEK-293 cells. We produced a modified Nav1.9 channel to follow its distribution

within the cytoplasm and the plasma membrane by TIRF imaging as it was previously described for Nav1.6. A similarly modified Nav1.7 channel, known to produce robust current in HEK-293, was used as a comparator. We showed that very low number of Nav1.9 channels were present on the surface of most HEK-293 cells after transient transfection. However, only a subtle difference was observed in total protein expression between Nav1.9 and Nav1.7 constructs on a Western blot suggesting impaired delivery of Nav1.9 to the cell surface. To look for possible explanations, we focused on C-terminal part of the channel. A chimeric construct with C-terminus of Nav1.9 replaced with C-terminus of Nav1.7 produced a substantial current in transiently transfected HEK-293 cells, although its electrophysiological properties were considerably altered compared to the native Nav1.9 channel.
We next explored the role of Nav C-termini in functional expression of Nav1.9 in HEK-293 cells by creating a number of constructs carrying C-termini of either Nav1.9, or Nav1.7, or Nav1.6, or a full GFP sequence (as a control). Surprisingly, expression of Nav1.9 C-terminus in isolation was dramatically lower in HEK-293 cells compared to all other C-termini and GFP. Substitution analysis in the C-terminus of Nav1.9 by the corresponding sequence from Nav1.7 allowed us to narrow down the critical expression determinant to 49 amino acids.
Finally, we tested the role of the newly identified 49 amino acids in the contest of the full channel length. Although steady-state levels of the protein were not substantially increased as measured on western blots, chimeric construct Nav1.9-$49_{Nav1.7}$ produced substantial current in transiently transfected HEK-293 cells while preserving most of electrophysiological properties of the native Nav1.9 channel. The reciprocal chimera Nav1.7-$49_{Nav1.9}$ showed a reduction in functional Nav1.7 expression accompanied by alterations of channel electrophysiological properties. Altogether, these data suggest a critical role of the newly identified 49 amino acid stretch in channel functional expression.

Disclosures: D.V. Sizova: None. **J. Huang:** None. **E.J. Akin:** None. **S.G. Waxman:** None. **S.D. Dib-Hajj:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.15/DP02/B57

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: B.04. Ion Channels

Support: NIH Medical Scientist Training Program
Center Grant B9253-C from the U.S. Department of Veterans Affairs Rehabilitation Research and Development Service and the Center for

Neuroscience and Regeneration Research, a Collaboration of the Paralyzed Veterans of America with Yale University.

Title: Real-time imaging of $Na_v1.7$ and $Na_v1.8$ subcellular distribution, trafficking, and membrane dynamics in sensory neurons

Authors: *G. HIGERD[1,2,3], E. J. AKIN[2,3], M. ALSALOUM[1,2,3], F. DIB-HAJJ[2,3], S. LIU[3,2], S. G. WAXMAN[2,3], S. D. DIB-HAJJ[2,3];
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Abstract: Voltage-gated sodium channels (Navs) are responsible for the rising phase of the action potential and play a critical role in regulating neuronal excitability. In particular, Navs 1.7 and 1.8 are expressed in dorsal root ganglion (DRG) sensory neurons and largely determine the excitability of these cells. Mutations in these channels are implicated in genetic pain disorders, with gain-of-function mutations leading to uncontrollable pain in inherited erythromelalgia, and loss-of-function in Nav1.7 to congenital insensitivity to pain. As key transducers of pain signals, these channels are promising targets for non-opioid analgesia. While the electrophysiological properties of these channels and the effects of disease-causing mutations have been characterized, little is known regarding their subcellular localization and dynamic organization in nerve cells. Here, we describe studies using HaloTag and SNAPTag self-labeling protein tags, together with bright and photostable Janelia Fluor-tagged Halo and SNAP ligands, to visualize full-length tagged sodium channels in live neurons at single molecule resolution. We are using tagged channels to investigate possible isoform-specific differences in cell surface expression on soma as well as proximal and distal axonal membranes. Using single particle tracking microscopy, we are investigating the stability of these channels within the plasma membrane in the distal axon. Using a novel optical pulse-chase assay in microfluidic chambers, Optical Pulse-Chase Axon Long-distance (OPAL) imaging, we are investigating axonal trafficking of Nav1.8 and Nav1.7 in vesicles. We are also investigating the effects of inflammatory mediators on the trafficking of these channels. These studies will advance our knowledge regarding possible isoform-specific channel distribution and dynamics in sensory axons at a distance from the soma, which will add to our understanding of the development and maintenance of excitable membranes and help to inform the design of new analgesic therapies.

Disclosures: G. Higerd: None. **E.J. Akin:** None. **M. Alsaloum:** None. **F. Dib-Hajj:** None. **S. Liu:** None. **S.G. Waxman:** None. **S.D. Dib-Hajj:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.16/B58

Topic: B.04. Ion Channels

Title: Profiling of subtype and state-dependent inhibitory effects of several analgesics and compounds under development on voltage-gated sodium channels

Authors: *T. OCHIAI[1], T. TABATA[1], S. KOYAMA[2], S. MIHARA[1], M. MICHISHITA[1], S. YOSHIKAWA[1];
[1]Asahi Kasei Pharma, Izunokuni-city, Japan; [2]Lab. for Pharmacol., Asahi Kasei Pharma Corp., Izunokunishi, Japan

Abstract: A significant unmet medical need remains in the area of chronic pain, and the generation of a novel analgesics is desired all over the world. It is well known that voltage-gated sodium (Nav) channels primarily contribute to the generation of neuronal action potentials, and it is thought that they play a pivotal role in the pathogenesis of pain. In fact, it is known that several drugs such as lidocaine, non-selective Nav channel blocker, show potent analgesic efficacy in clinic. It has been also reported that Nav channels have multiple states such as resting state (RS), fast inactivation state (FIS), slow inactivation state (SIS), however there is still room for discussion about a preferable subtypes and states-selectivity for the drug discovery of novel analgesics targeting Nav channels. We have recently developed an electrophysiological assay platform for Nav channels internally, and initially evaluated state-dependent inhibitory profiles of clinically used analgesics known to inhibit Nav channels (carbamazepine, lidocaine, mexiletine, and duloxetine), CNV-1014802 (under clinical trial), and PF-05089771 (previously under clinical trial) on Nav1.3, Nav1.6, Nav1.7, Nav1.8 (those are from efficacy viewpoint), and Nav1.5 (safety viewpoint). In our experiments, sodium currents were measured by Automated Patch Clamp Systems in the presence of compounds using CHO cells expressing human(h) Nav1.3, Nav1.5, Nav1.6, Nav1.7, or Nav1.8. In each assay protocol, RS current was measured by applying a test pulse of 10 mV for 16.5 ms at a holding potential of -120 mV. FIS and SIS were induced by applying pre-pulses, and currents were measured by a test pulse of 10 mV for 50 ms in the condition that about half of Nav channels were inactivated. As a result, carbamazepine was shown to be non-state-selective, lidocaine was FIS-selective, mexiletine was FIS and SIS-selective, and duloxetine was SIS-selective fashion, respectively. CNV-1014802 showed high potency for both FIS and SIS of hNav1.7 and hNav1.8. It also showed high potency for FIS of hNav1.5 in our study. PF-05089771 showed higher potency for all states of hNav1.7 than for other subtypes; it was also newly found that the potency of PF-05089771 for hNav1.7 SIS was stronger than for hNav1.7 FIS. In this presentation, we will discuss a future direction of drug discovery targeting Nav channels for the treatment of chronic pain based on the above data.

Disclosures: **T. Ochiai:** None. **T. Tabata:** None. **S. Koyama:** None. **S. Mihara:** None. **M. Michishita:** None. **S. Yoshikawa:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.17/B59

Topic: B.04. Ion Channels

Support: Columbia TFFI Award

Title: Sodium channels play an important role in vincristine-induced painful neuropathy

Authors: ***L. CHEN**, J. HUANG, C. GOMIS-PEREZ, C. BENSON, P. EFFRAIM, S. D. DIB-HAJJ, S. G. WAXMAN;
Yale Univ., New Haven, CT

Abstract: Vincristine, a widely used chemotherapeutic agent, produces painful peripheral neuropathy in patients. The underlying mechanisms are not well understood. In this study, we investigated the involvement of voltage-gated sodium channels in a mouse model of vincristine-induced painful neuropathy. We found that vincristine treatment (0.75 mg/kg twice a week for four weeks) results in the development of thermal and mechanical allodynia and renders small neurons significantly hyper-excitable with both reduced current threshold and increased firing frequency. Histological examinations did not reveal structural changes at proximal sciatic nerve or distal toe nerve at the vincristine dose used in this study. Immunohistochemical studies and *in vivo* imaging also confirmed that there is no significant change in density or morphology of intra-epidermis nerve terminals. Voltage-clamp recordings of small DRG neurons from vincristine-treated animals showed a 3 mV hyperpolarizing shift in V1/2 of TTX-S current activation and an 8 mV hyperpolarizing shift in V1/2 of Nav1.8 activation. These changes in biophysical properties of sodium currents likely contribute to the enhanced action potential firing observed in small neurons. Our data show that TTX-S sodium channels (most likely Nav1.7) and TTX-R sodium channel Nav1.8 together are involved in the hyper-excitability of small DRG neurons following vincristine treatment which underlies the development of vincristine-induced painful neuropathy.

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Poster

035. Sodium Channels in Health and Disease

Location: Hall A

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Program #/Poster #: 035.18/B60

Topic: B.04. Ion Channels

Support: UTMB Presidential Scholars Program (CMT,KEM)
NIH/NIEHS-T32ES007254 (CMT)
NIEHS Center Grant-P30ES006676 (FL)

Title: Early-life exposure to a pyrethroid insecticide results in electrophysiological and behavioral aberrations

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Abstract: Deltamethrin (DM), a commonly used pyrethroid insecticide, exerts its effect on insects by delaying onset of inactivation in voltage gated sodium (Nav) channels fundamental for neuronal excitability. Epidemiological data showed a correlation between pyrethroid metabolites in urine and increased risk of attention-deficit/hyperactivity disorder (ADHD) diagnosis in children. In rats, exposure to DM results in behavioral phenotypes that mimic aspects of ADHD and are associated with the dopaminergic (DA) reward pathway in the nucleus accumbens (NAc). Dysregulation of one subtype of the predominate neuron of the NAc, D1 medium spiny neurons (MSNs), has been implicated in multiple neurodevelopmental disorders such as ADHD and autism spectrum disorder. In these MSNs, there is also a prevalence of the isoform Nav channel 1.6 which in heterologous cell studies conducted in our laboratory was shown to be altered by prolonged DM exposure. Here, we investigate behavioral aberrations and subtype specific MSNs dysfunction following early-life exposure to a low level of DM. For the early-life exposure model, pregnant female C57BL/6J mice were exposed to 3.0 mg/kg of DM throughout pregnancy and lactation. Then, male mice litter-mates from post-natal day ~30 were used for subsequent experiments. A variety of behavioral assays were conducted on these animals. In the open field test total distance traveled was increased, in the social interaction assay time mobile in the interaction zone was decreased and in the novel object recognition assay time spent in both the novel and familiar zones was decreased for mice exposed to DM (n=10-17, two sample t-test, $p<0.05$). We also employed whole-cell patch-clamp electrophysiology in coronal brain slices to monitor changes in NAc MSNs firing due to early-life DM exposure. A decrease in the total number of action potentials and instantaneous firing frequency was observed (n=7-12, two-sample t-test, $p<0.05$). We are currently conducting further electrophysiological experiments to

determine changes to specific MSN subtypes utilizing a transgenic mouse line with MSN subtype specific fluorescent labeling and post-hoc analysis of electrophysiological parameters. Together, these studies illustrate the lasting effects of early-life DM exposure on MSNs in the NAc and behavioral aberrations. These studies will advance our knowledge of the toxic activity of DM in the developing brain and help assess risk exposure in the human population and the increased vulnerability to neurodevelopmental disorders.

Disclosures: C.M. Tapia: None. **K.E. McDonough:** None. **L.M. Hallberg:** None. **B.T. Ameredes:** None. **T.A. Green:** None. **F. Laezza:** None.

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.19/B61

Topic: B.04. Ion Channels

Title: Preclinical candidate DWP17061, a novel Nav1.7 blocker, suppresses nocifensive behavior in pain animal models

Authors: *S.-Y. KIM, J. KANG;
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Abstract: Voltage-gated sodium channels are one of the major players in generating and propagating action potentials. Nav1.7 is usually expressed at high levels in the nociceptive (pain) neurons at dorsal root ganglion (DRG). Gain of function of Nav1.7 channel leads to result in extreme pain in humans, but loss of function of it leads to Congenital Inability to Pain (CIP). But, in spite of genetic validation, Nav1.7 blockers have not been successful in clinical trials.Here we characterized DWP17061 as potent and selective Nav1.7 blocker *in vitro* and *in vivo* pain models. DWP17061 was superior to PF-05089771 in PK profiles and efficacy. We expect DWP17061 to be a successful analgesic.

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

Poster

035. Sodium Channels in Health and Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 035.20/B62

Topic: B.04. Ion Channels

Support: NIH grants R01MH080234

Title: A mouse model of schizophrenia risk gene SETD1A displays neuronal hyper-excitability attributable to enhanced persistent sodium current

Authors: *G. W. CRABTREE[1], J. A. GOGOS[1,2];
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Abstract: We have recently described the identification of a novel schizophrenia susceptibility gene, the histone methyl transferase SETD1A. A mouse model carrying a loss-of-function (LoF) mutation in the mouse orthologue shows alterations in axon branching, cortical neurophysiology, and cognitive function. To determine potential sources of neuronal dysfunction in Setd1a(+/-) mice relevant to schizophrenia, we performed whole-cell recordings in acute brain slices of prelimbic/infralimbic medial prefrontal cortex (mPFC) from layer 2/3 pyramidal neurons. Whole-cell current-clamp recordings in from layer 2/3 pyramidal neurons revealed enhanced excitability in Setd1a(+/-) mice. Parallel cell-attached recordings from layer 2/3 pyramidal neurons revealed alterations in the population distributions of neurons displaying spontaneous action potentials that were consistent with hyper-excitability and further suggested Setd1a(+/-) hyper-excitability is likely impactful under physiologically relevant network conditions. Notably, Setd1a(+/-) neurons did not differ from WT in resting membrane potential, membrane input resistance, or membrane time constant suggesting hyperexcitability arose due to alteration in active conductances. Subsequent voltage-clamp recordings employing voltage-steps revealed that current responses in Setd1a(+/-) neurons differed from WT only over a very narrow voltage range encompassing typical action potential threshold voltages. In this voltage range, Setd1a(+/-) neurons were deficient in net outward, hyperpolarizing currents consistent with the observed hyper-excitability. Further recordings employing slow (100mV/s) voltage ramps revealed that this difference arose due to marked enhancement in Setd1a(+/-) mice of the persistent sodium current that arises from incomplete inactivation of the “fast” voltage-gated sodium channels principally involved in action potential generation. Taken together, these findings indicate dysregulation of the persistent sodium current in Setd1a(+/-) mice is responsible for observed neuronal hyper-excitability and further suggest similar dysfunction could play a broader role in schizophrenia in general. This work was supported by NIH grant R01MH080234.

Disclosures: G.W. Crabtree: None. **J.A. Gogos:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.01/B63

Topic: B.05. Neurotransmitter Release

Support: University of Lille 1, Sapienza University of Rome (Framework Agreement signed on February 15th, 2007), and the CNRS within the framework of the Prenatal Stress and Neurodegenerative Diseases International Associated Laboratory (LIA-PSND).

Title: Sex-related effects of perinatal stress on the glutamatergic synapse and related behaviors in aged rats

Authors: R. VERHAEGHE[1,2], ***S. MORLEY FLETCHER**[1,3], H. BOUWALERH[1,3], G. VAN CAMP[1,3], F. NICOLETTI[4,5], S. MACCARI[1,6];
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Abstract: Perinatal stress (PRS) causes impairment of glutamate release in the hippocampus and changes in glutamate-related behaviors, including a reduction in risk-taking behavior, in adult rats. Here, we examined whether similar changes occurred during aging using the offspring of dams exposed to repeated episodes of restraint stress during pregnancy (here indicated as "PRS rats"). We used 21-months old PRS rats and their controls of both sexes considering that sex is an important variable in mechanisms of resilience to stress and in the vulnerability to stress-related disorders. Aged male PRS rats exhibited decreased risk-taking behavior, spatial memory, and gross and thin motor skills. In contrast, aged female PRS showed reduced risk-taking behavior but no changes in spatial memory and gross or thin motor skills. Similarly, to adult PRS rats, these behavioral alterations were associated with large reductions in the levels of synaptic vesicle-related proteins (Rab3a, SNAP25, and syntaxin) in the ventral hippocampus. Of note, synaptic vesicle-related proteins were also reduced in the dorsal hippocampus and striatum. In contrast, aged female PRS rats did not show alterations in the expression of synaptic vesicle-related proteins in the three brain regions. Adult and aged PRS rats of both sexes showed a reduction of mGlu2/3 receptor protein levels in the ventral hippocampus, whereas mGlu2/3 receptor levels were reduced in the prefrontal cortex and striatum exclusively during aging. These results suggest that early life stress causes life-long and gender-dependent alterations in glutamatergic transmission and related behaviors. This might be relevant in the pathophysiology of stress-related disorders during aging.

Disclosures: R. Verhaeghe: None. **S. Morley fletcher:** None. **H. Bouwalerh:** None. **G. Van Camp:** None. **F. Nicoletti:** None. **S. Maccari:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.02/B64

Topic: B.05. Neurotransmitter Release

Support: University of Lille 1, Sapienza University of Rome (Framework Agreement signed on February 15th, 2007), and the CNRS within the framework of the Prenatal Stress and Neurodegenerative Diseases International Associated Laboratory (LIA-PSND).

Title: Early life stress causes a long-lasting dopaminergic synaptopathy in the nigro-striatal system and related behavioral dysfunction

Authors: *S. MACCARI[1,2], S. MORLEY FLETCHER[3,4], J. MARROCCO[5], R. VERHAEGHE[6,1], G. VAN CAMP[3,4], H. BOUWALERH[3,4], D. BUCCI[7], M. CANNELLA[7], A. PITTALUGA[8], G. BATTAGLIA[6,7], F. NICOLETTI[6,7];
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Abstract: Comorbidity exists between stress-related disorders and disorders of the extrapyramidal motor system. We decided to examine how perinatal stress influences neurochemical and behavioral parameters related to striatal motor function. We found that 4-month old PRS rats (i.e., the adult offspring of dams exposed to multiple episodes of restraint stress during pregnancy causing reduced maternal care) showed a reduction in depolarization-evoked dopamine (DA) release in the corpus striatum, as assessed by *in vivo* microdialysis and measurement of ^{3}H-DA efflux from superfused isolated synaptosomes, associated with an increase in steady-state DA levels in the striatum. The number of tyrosine hydroxylase $(TH)^{+}$ cells in substantia nigra and TH protein levels in striatal synaptosomes was reduced in adult PRS, whereas striatal levels of the high-affinity DA transporter (DAT) were unchanged. As a behavioral correlate of these findings, adult PRS rats showed a defective striatal motor performance in the grip strength and pasta matrix reaching tests. We extended the analysis to aged (20-month old) PRS rats, which also showed a reduction in the evoked release of ^{3}H-DA

from striatal synaptosomes and a defect in striatal motor function (pasta matrix, and ladder rung walking tests) with respect to age-matched unstressed controls. We analyzed different dopaminergic synapse-related proteins in the striatum of aged rats, finding no changes in TH, DAT, and D1 or D2 receptors, whereas, interestingly, α-synuclein protein levels were increased. Moreover, in aged PRS rats, we observed a reduction in syntaxin, Rab3a and VAMP synaptic vesicle-related proteins in the striatum. Our findings suggest that early life stress may cause abnormalities in nigrostriatal dopaminergic function and related motor performance, which are seen in both adult and aged rats.

Disclosures: **S. Maccari:** None. **S. Morley fletcher:** None. **J. Marrocco:** None. **R. Verhaeghe:** None. **G. Van Camp:** None. **H. Bouwalerh:** None. **D. Bucci:** None. **M. Cannella:** None. **A. Pittaluga:** None. **G. Battaglia:** None. **F. Nicoletti:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.03/B65

Topic: B.05. Neurotransmitter Release

Support: Spanish MINEICO BFU2016-76050-P; BFU2013-47493-P; BES-2014-070405
ISCIII CIBERNED (PRY-18-606, PI2015-2/06-1)
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FEDER
Iniciativa de Empleo Juvenil (Fondo Social Europeo)

Title: Dysfunctional synaptic transmission mediated by parvalbumin (PV) cortical interneurons in hyperactive PV^{cre}conditional CSPalpha/DNAJC5 KO mice

Authors: M. VALENZUELA-VILLATORO, P. GARCIA-JUNCO-CLEMENTE, J. L. NIETO-GONZALEZ, S. LOPEZ-BEGINES, M. C. RIVERO, F. MAVILLARD, ***R. FERNANDEZ-CHACON**;
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Abstract: Cysteine String Protein (CSPα/DNAJC5) is a synaptic co-chaperone that prevents activity-dependent degeneration of synapses formed by fast-spiking parvalbumin-positive (PV) basket cells. Based on a mouse line bearing a *Dnajc5* floxed allele (Nieto Gonzalez et al., Proc. Natl. Acad Sci. USA, 2019) we have now conditionally targeted *Dnajc5* in PV+ GABAergic neurons (PV^{cre}:Ai27D:$Dnajc5^{flox}$mice). These mice suffer from hyperactivity, dystonia and ataxia but without increased mortality compared to control mice at least up to 8 months of age. We have analyzed the intrinsic neuronal properties and the synaptic function of PV interneurons

at the motor cortex (layer II/III). The intrinsic neuronal properties and excitability are similar in interneurons lacking CSPα/DNAJC5 compared to controls at 2 months of age. At 8 months of age, we detected changes in rheobase and input resistance between interneurons with and without CSPα/DNAJC5, however, the number and size of PV somata were not reduced. Next, we analyzed spontaneous release of GABA onto pyramidal neurons and found that the frequency and amplitude of miniature inhibitory currents (mIPSC) were reduced in mutant mice. The mIPSC frequency was reduced at 8 months compared to 2 months, which is consistent with progressive synaptic degeneration in the absence of CSPα/DNAJC5. Remarkably, the mIPSC amplitude was equally reduced at both ages which it might reveal a primary deficit in the vesicular GABA content and/or secondary postsynaptic changes in GABA receptors. These observations set-up an interesting scenario potentially unvealing a dual role for CSPα/DNAJC5 to operate and to maintain synaptic function. Future experiments will investigate the molecular connection between both roles for CSPα/DNAJC5 in PV cortical interneurons. We are grateful to A. Arroyo Saborido and C. Cabrera Romero for excellent technical assistance.

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Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.04/B66

Topic: B.05. Neurotransmitter Release

Support: NIH GM111997 (AA)

Title: A role for synaptotagmin-7 in plasticity of the sympathoadrenal synapse

Authors: R. N. CABALLERO[1], J. PHILIPPE[1], M. BENDAHMANE[1], A. CHAPMAN-MORALES[2], A. ANANTHARAM[3], *P. M. JENKINS[4];
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Abstract: Synaptotagmin-7 (Syt7) is one of the major calcium sensors for regulated exocytosis in the central and peripheral nervous system. Its high sensitivity allows tunable secretory responses to a range of stimuli that result in graded increases in intracellular calcium. Despite the importance of Syt7, questions remain as to its localization and specific functions in nervous tissue. Here, these issues were examined at a key effector arm of the sympathetic nervous system - the adrenal medulla - with mice lacking endogenous Syt7 (Syt7 KO). First, using immunohistochemistry on frozen sections of adrenal glands, we find Syt7 in the axons and

terminals of neurons innervating medullary chromaffin cells, as well as in chromaffin cells themselves. Syt7 is punctate in appearance consistent with its sorting to organelles. Using whole cell electrophysiology in adrenal slices, we find that Syt7 is required for a form of synaptic enhancement termed pair-pulse facilitation as well as for tonic current after a train of pulses. Syt7 null neurons also more readily undergo depression in response to repeated depolarization than WT neurons. Our study shows that Syt7 serves key functional roles as a regulator of neurotransmitter release at pre-synaptic terminals in the peripheral nervous system. In addition, our results provide new evidence that Syt7 is acting at the presynaptic terminal in the peripheral nervous system.

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Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.05/B67

Topic: B.05. Neurotransmitter Release

Support: ERC Advanced Grant 694539

Title: Modulation of neurotransmitter release via KCTDs at the medial habenula to interpeduncular nucleus pathway

Authors: P. BHANDARI[1], D. VANDAEL[1], T. FRITZIUS[2], D. KLEINDIENST[1], M. GASSMANN[2], A. KULIK[3], P. JONAS[1], B. BETTLER[2], R. SHIGEMOTO[1], ***P. KOPPENSTEINER**[1];
[1]IST Austria, Klosterneuburg, Austria; [2]Dept. of Biomedicine, Univ. of Basel, Basel, Switzerland; [3]Inst. of Physiol. II, Freiburg, Germany

Abstract: The medial habenula (MHb) to interpeduncular nucleus (IPN) pathway has been recently implicated in the modulation of aversive memory. This neuronal circuit exhibits several rare properties, the most striking of which is the presence of voltage-gated calcium channel 2.3 (Cav2.3) at the presynaptic MHb terminal. In addition, presynaptic $GABA_B$ receptors, which mediate inhibitory signaling in many brain areas, may exert an unusual excitatory effect on neurotransmitter release from MHb terminals. However, the mechanism underlying this excitatory action of $GABA_B$ receptors is unknown. To better understand the modulation of Cav2.3-mediated release via $GABA_B$ receptors in this pathway, we studied the co-localization of presynaptic molecules on MHb terminals using quantitative SDS-digested freeze-fracture replica labeling (SDS-FRL) and examined the functional implications of molecular interactions via *in vitro* electrophysiology. We found Cav2.3 molecules concentrated in the presynaptic active zone

of MHb terminals in SDS-FRL and confirmed that vesicular neurotransmitter release relies exclusively on Cav2.3. Furthermore, we discovered prominent co-clustering of presynaptic Cav2.3 with $GABA_B$ receptors and their auxiliary subunits, KCTD8 and KCTD12b. In a co-immunoprecipitation experiment in HEK cells, we found specific binding of Cav2.3 to KCTD8 and KCTD12b, but not to KCTD12. Using variance-mean analysis, we observed a significant increase in the probability of neurotransmitter release in mice lacking KCTD12b. In contrast, release probability was reduced in KCTD8 KO mice. However, the absence of neither KCTD8 nor KCTD12b interfered with the $GABA_B$ receptor-mediated enhancement of neurotransmitter release from MHb terminals. Although the mechanism underlying the excitatory effect of $GABA_B$ receptors remains unclear, our study suggests the interaction of Cav2.3 with KCTDs as a novel mechanisms by which synaptic strength may be scaled at the MHb-IPN pathway.

Disclosures: P. Bhandari: None. **D. Vandael:** None. **T. Fritzius:** None. **D. Kleindienst:** None. **M. Gassmann:** None. **A. Kulik:** None. **P. Jonas:** None. **B. Bettler:** None. **R. Shigemoto:** None. **P. Koppensteiner:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.06/B68

Topic: B.05. Neurotransmitter Release

Title: Quantitative studies of autonomic nervous system activities in urinary bladder smooth muscle cells towards bladder overactivities

Authors: *C. MAHAPATRA, R. MANCHANDA;
Bio Sci. & Bio Engin., Indian Inst. of Technol. Bombay, Mumbai, India

Abstract: *Context:* The urinary incontinence (UI) is defined as the involuntary loss of urine and associated with the enhanced spontaneous contractions of the detrusor smooth muscle (DSM). The spontaneously evoked action potentials (sAPs) in DSM cells initiate and modulate these contractions. The DSM is strongly innervated, connecting approximately 16000 afferent and efferent axons from ganglion neurons. It generates sAPs due to the stochastic nature of purinergic neurotransmitter release from the parasympathetic nerve. *Objectives:* The aim of this current study is to understand the putative relationship between the fluctuating ion channel conductances and stochastically release of ATP in generating sAPs.*Methods:* The neurotransmitter current was considered as an independent excitatory conductance in the model where $g_{ex}(t)$ and $E_{ex}(t)$ are the one-variable stochastic process conductance and the reversal potential respectively. In addition, D_{ex} and $\lambda_1(t)$ are known as the diffusion coefficients and Gaussian white noise. The point-conductance is incorporated into a single DSM cell model based on a single cylindrical compartment. *Results*: The elicited AP consists an after depolarization and

after hyperpolarization phase. The AP peak amplitude and duration are about 5 mV and 40 ms respectively. Then, the random injection of the point process model is conducted to elicit a series of sAPs and depolarization for 5 seconds. The membrane resting potential is held at ─ 50 mV with a 3 mV of fluctuation. The stochastically depolarization up to 20 mV activates the T-type Ca^{2+} channel first and then the L-type Ca^{2+} channel to generate an action potential. *Conclusions***:** The T-type Ca^{2+} channel blocker can be used as a new pharmacological target for UI. In addition, extended multidimensional models will aid our understanding of DSM electrical and contractile function, providing windows of insight into the factors that govern excitability and the contraction in both normal and unstable bladder, in turn shedding light on such phenomena as bladder overactivity and its underlying mechanisms.

Disclosures: C. Mahapatra: None. **R. Manchanda:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.07/B69

Topic: B.05. Neurotransmitter Release

Title: Regulation of brain synapses and behaviour by miR-138

Authors: *R. R. DASWANI, G. SCHRATT;
ETH, Zuerich, Switzerland

Abstract: MicroRNAs are small non-coding RNA molecules playing an important role in fine tuning gene expression. They have been implicated in all aspects of development, physiology and higher cognitive processes of the nervous system. The mechanisms underlying microRNAs function in the brain have not been fully clarified yet, particularly *in vivo*. MiR-138, a brain enriched microRNA, negatively regulates dendritic spine size and miniature Excitatory Post-Synaptic Currents in hippocampal primary neurons. We established a mouse line to study the *in vivo* miR-138 loss of function. Whole hippocampal RNA sequencing and subsequent GO TERM analysis show an abundance of differentially regulated synaptic genes after miR-138 loss of function. Next, by electrophysiological field recordings, we show impaired synaptic transmission most likely due to a pre-synaptic machinery defect in the Schaffer Collaterals. Consistently, no morphological abnormalities in dendritic spines have been detected by Golgi staining of hippocampal slices. Preliminary behavioural tests suggest that these pre-synaptic impairment leads to defect in short term memory. Taken together these data suggest that miR-138 is a crucial regulator of pre-synaptic release in hippocampal area with a potential impact on working memory in rodents.

Disclosures: R.R. Daswani: None. **G. Schratt:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.08/B70

Topic: B.05. Neurotransmitter Release

Support: NIH MH084874
NIH MH101672

Title: Lattice light sheet microscopy reveals presynaptic Ca^{2+} transients highly variant in amplitude and Ca^{2+} channel subtype

Authors: *S. RODRIGUEZ[1], M. POTCOAVA[2], S. RAMACHANDRAN[4], S. T. ALFORD[3]; [1]Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL; [2]Anat. and Cell Biol., [3]Dept. of Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL; [4]Biol. Sci., Univ. Illinois, Chicago, Chicago, IL

Abstract: Transmitter release is initiated by action potential (AP)-evoked Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs). Various spatio-temporal Ca^{2+} requirements for release have been proposed in different presynaptic terminals, by Ca^{2+} influx through different numbers of VGCCs with varying spatial relationships to one another and to the release machinery. VGCCs may be clustered in domains or be more diffuse with perhaps only one channel necessary for release. The number of functional VGCCs in central synapses is nevertheless small. This implies that variability of Ca^{2+} transients between APs is high. However, variability is difficult to measure because VGCCs and terminals are inaccessible to direct recording approaches and imaging lacks resolution to determine Ca^{2+} entry at active zones. Using an acute axon dissociation from lamprey we recorded from presynaptic terminals devoid of apposing postsynapses. Active zone VGCCs were characterized by single channel recordings. N, P/Q, and R-type were all present with small numbers (3-6, mean 4) of channels opened on single stimuli. Imaging of the resultant presynaptic Ca^{2+} transients responsible for neurotransmitter release requires high speed and sensitivity. Speed has been achieved in a number of preparations using confocal or multiphoton line-scanning or similar approaches. However, high excitation intensities and limited resolution makes an assessment of signal reliability difficult, while wide field epifluorescence imaging in tissue creates excessive noise from out-of-focus information. Lattice light sheet (LLS) imaging overcomes many of these limitations. It enables low-light excitation and thin sheet (400 nm) excitation through active zones. Using an adapted LLS microscope, we imaged Ca^{2+} entry to lamprey giant axons, at up to 1kHz in single planes through axons labeled with Ca^{2+} dyes. This reveals activation of multiple active zones. Ca^{2+} signals demonstrated quantal-like fluctuations in amplitude randomized across different active zones in single axons. On repetitive stimulation, evoked transients showed frequency and Ca^{2+} dependent

depression in amplitudes. Similar to data from cell attached recordings, N, P/Q and R-type channels were pharmacologically identified, but contributions of individual channel types to the signal varied between active zones in the same axons.

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Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.09/B71

Topic: B.05. Neurotransmitter Release

Support: Grant-In-Aid for Science Research (C), JSPS, KAKENHI 17K08501

Title: Morphological and molecular characteristics of renal sympathetic nerve endings attached to multi-effector modules

Authors: *S. MAEDA[1], M. FUJIHIRA[1], H. HORI[1,2], Y. MINATO[1], S. KUWAHARA-OTANI[1], H. YAGI[1];
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Abstract: Renal sympathetic nerves are innervated to several effectors, such as vascular smooth muscle cells, tubular epithelial cells, and juxtaglomerular cells, each of which have a unique sensitivity to norepinephrine (NE). However, the detailed mechanism by which renal nerves differentially stimulate the effectors remains unclear. In this study, we immunohistochemically examined renal nerve terminal characterizations, and expression of specific adhesive molecule integrins in terminal-effector contacts in the rat kidney. Tyrosine hydroxylase (TH) positive neurons were passed along to the interlobular and afferent arterioles, and innervated to the urinary tubules with varicosities. Single-labeled renal ganglion nerve fiber with td-Tomato plasmid was co-bundled with TH positive nerves and projected to the effectors. These terminal varicosities were covered with S-100$^+$ Schwann cells and closely attached to the outer basal lamina of the effector cells. Immunoelectron microscopy showed that each terminal covered with S-100$^+$ Schwann cells was separated from the other effectors in the narrow interstitium of the renal tubules. This suggests that renal nerve varicosity terminals may release NE to the definite effectors intensively, but not diffusely in the interstitium. Additionally, to examine the adhesion molecules that maintained nerve terminals with the basal lamina of the effectors, several integrin subtypes in the renal cortical nerves were subjected to immunohistochemistry. The integrin α4 subunit was expressed in the TH$^+$ renal nerves and colocalized with synaptophysin1. Because integrin α4 is composed of a heterodimer with integrin β1 or β7, these integrins were examined

immunohistochemically. Results showed that integrin β1 was distributed in the renal nerve fibers. Localization analysis of integrin α4 and fibronectin at nerve terminals revealed that they were closely adjacent to each other, suggesting that the integrin heterodimer α4β1 (known as VLA-4) may be one of the attachment molecules predicted to support the terminal junctions.

Disclosures: **S. Maeda:** None. **M. Fujihira:** None. **H. Hori:** None. **Y. Minato:** None. **S. Kuwahara-Otani:** None. **H. Yagi:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.10/B72

Topic: B.05. Neurotransmitter Release

Support: Shanghai Municipal Science and Technology Major Project 18JC1410100
Shanghai Municipal Science and Technology Major Project 2018SHZDZX05
Key Research Program of Frontier Sciences of Chinese Academy of Sciences QYZDY-SSW-SMC028

Title: Logic of the locus coeruleus-norepinephrine system in anatomical and functional organization

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Abstract: The Locus Coeruleus (LC) system in the brainstem spreads extensive axonal projections to almost all brain areas and modulates diverse neural functions. It has been a long-standing mystery whether LC neurons work synergistically or with heterogeneous modularity. Here, using the larval zebrafish as a model, we first labeled and reconstructed the morphology of individual LC neurons, and found that that each LC neuron projects broadly from the forebrain to spinal cord and shows distinct and asymmetrical projection with ipsilateral preference. In contrast, the axonal projection of the whole LC system is symmetrical and evenly ramifies in terminal regions. Interestingly, different LC neuron displays similar physiological properties by electrophysiology recording. Further, we found all of LC neurons display synchronized spontaneous phasic firing and consistent sensory evoked responses. NE releases detected by NE sensor in vivo show synchronization and homogeneity in LC terminal regions. To further assess the role of the LC homogeneity on network activities, we ablated LC neurons and found that the excitability and correlation of
brain-wide spontaneous activities decreased significantly, suggesting that LC functional

homogeneity is required for coordinating brain-wide neuronal activities. The study comprehensively reveals the basic principle of LC system organizations.

Disclosures: C. Zhang: None. **Y. Yang:** None. **F. Li:** None. **J. Du:** None.

Poster

036. Presynaptic Organization and Transmitter Release

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 036.11/B73

Topic: B.05. Neurotransmitter Release

Title: Role of agmatine in the modulation of dopamine output in the rat ventral hippocampus

Authors: L. BETANCOURT[1], J. URBANAVICIUS[2], S. FABIUS[2], M. RAMIREZ[1], P. RADA[1], C. SCORZA[2], *L. HERNANDEZ[1,3];
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Abstract: Agmatine (AGM) is an endogenous arginine metabolite that acts as neuromodulator, involved in a broad spectrum of central nervous system (CNS) diseases. AGM seems to act as an endogenous ligand for α_2 adrenergic and imidazoline receptors but it has also been proposed as an N-methyl-D-Aspartate (NMDA) receptor antagonist. It has recently been shown that local infusion of this metabolite enhances extracellular dopamine (DA) levels in the striatum, effect that was similar to that one reported for the non-competitive NMDA-R antagonists like phencyclidine (PCP), ketamine and MK-801, agents commonly used as pharmacological tools to model schizophrenia. Additionally, acute administration of PCP affects arginine metabolism in the brain and other research showed increased AGM concentration and glutamate/GABA ratio in frontal cortex and plasma from the schizophrenia cases. Hippocampus sends (HC) sends direct projections to the nucleus accumbens, or indirect projections to the entorhinal, cingulate and prefrontal cortices and has been involved in behaviors relevant to psychosis. The existence in the HC of a population of AGM synthetizing is well known, but it is still unknown if AGM modifies DA levels in this brain regions. Thus, the present study was aimed to explore this last possibility employing the *in vivo* microdialysis technique. For this purpose, AGM (43.8, 438 µM and 4.38 mM) was locally perfused by reverse dialysis (1.5 µl/min/20 min) in awake rats and DA levels in dialysate samples (30 µl) were analyzed and measured by HPLC with electrochemical detection. Results showed that AGM induced a dose-dependent increase in DA levels, a linear relationship was found in the range of doses 43.8 to 4.38 mM ($y = 29.25X + 122.6$; $R^2 = 0.997$) regression analysis R factor, $P < 0.001$. At 4.38 mM a significant and long-lasting (up to 80 min) increase on DA output was observed. Statistical analysis of extracellular [DA] revealed an overall significant effect of AGM ($F(3, 372.4)= 61.39$, $p < 0.05$) with all doses significantly different than saline. Our data suggest a role of endogenous AGM in the modulation of DA

neurotransmission in a brain region involved in the pathogenesis of schizophrenia and may lead to the future development of novel therapeutics for the disease.

Disclosures: L. Betancourt: None. **J. Urbanavicius:** None. **S. Fabius:** None. **M. Ramirez:** None. **P. Rada:** None. **C. Scorza:** None. **L. Hernandez:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.01/B74

Topic: B.06. Synaptic Transmission

Support: DAAD Scholarship for doctoral studies for Rachida Yakoubi and Prof. Joachim Lübke

Title: Ultrastructural heterogeneity of human layer 4 excitatory synaptic boutons in the adult temporal lobe neocortex

Authors: *R. YAKOUBI[1], A. ROLLENHAGEN[1], M. VON LEHE[2,3], D. MILLER[3], B. WALKENFORT[4], M. HASENBERG[4], K. SÄTZLER[5], J. LÜBKE[1,6,7];
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Abstract: Synapses are key structural elements underlying the computational properties of any network in the brain. Despite their important role, comparably little is known about these structures particularly, their quantitative morphology in humans.
To explore this aspect, we took advantage of non-affected neocortical access biopsy material, obtained, with consent, from three male and three female patients (25-63 years in age) who underwent epilepsy surgery to control the seizures. Experiments were approved by the ethic votum of Medical Faculty to Prof. Dr. med. Johannes Schramm and Prof. Dr. rer. nat. Joachim Lübke, Nr. 146/11), ethic votum of Medical Faculty to PD Dr. med. Marec von Lehe and Prof. Dr. rer. nat. Joachim Lübke, Reg. No. 5190-14-15; ethic votum of Medical Faculty to Dr. med. Dorothea Miller and Prof. Dr. rer. nat. Joachim Lübke, Reg. No. 17-6199-BR, and the EU directive (2015/565/EC and 2015/566/EC) concerning working with human tissue.
This material was used for high-end fine-scale electron microscopy (EM) and tomography, to provide the first comprehensive (coherent) quantitative study of layer 4 (L4) excitatory synaptic boutons (SBs) of the human temporal lobe neocortex (TLN). 3D-volume reconstructions of SBs were generated, the size of active zones (AZs) and that of the three functionally defined pools of synaptic vesicles (SVs) were particularly quantified.

SBs were comparably small (~2.50 μm^2 in surface area), predominantly containing a single AZ (~0.13 μm^2); preferentially established on spines of different types. The total pool was ~1800 SVs, although with a large variability (Min: 368 SVs; Max: 5053 SVs). The large total pool lead also to extremely large readily releasable (~20 SVs at a perimeter (p) of 10 nm and ~50 at p20 nm from the pre active zone), recycling (~ 80 SVs) and resting pools (~850 SVs). The results of EM tomography revealed also an average of 5.5 'docked' vesicles at individual pre active zones. The three pools of SVs in L4 SBs of the human TLN are 2 to 3-fold larger when compared with SBs of comparable or even larger size in various animal species. This constitutes a marked difference, among species, together with the large variability in the shape and size of AZs suggesting high synaptic efficacy and strength in synaptic transmission, but also marked short-term plasticity at L4 SBs.
Thus the specific structural composition of L4 SBs in the human TLN, underlie their function as 'amplifiers' of signals from the sensory periphery and in modulating synaptic activity in the human TLN, an important associative area involved in high-order brain functions such as audition, vision, memory, language processing, and various multimodal associations.

Disclosures: **R. Yakoubi:** None. **A. Rollenhagen:** None. **J. Lübke:** None. **M. von Lehe:** None. **D. Miller:** None. **B. Walkenfort:** None. **M. Hasenberg:** None. **K. Sätzler:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.02/B75

Topic: B.06. Synaptic Transmission

Support: JSPS/MEXT KAKENHI Grant 19H03331 to Y.F., 19K16269 to Y.M., 19K06893 to N.Y., 17H03678, 18H04873, 19H04974 to M.F.
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Daiko Foundation Grant to Y.F.
The Hori Sciences and Arts Foundation Grant to M.F.

Title: Physiological roles of trans-synaptic LGI1-ADAM22-MAGUK complex

Authors: *Y. FUKATA[1,3], Y. HIRANO[1,4], H. INAHASHI[1], Y. MIYAZAKI[1,3], N. YOKOI[1,3], M. SANBO[2], T. GOTO[2], M. HIRABAYASHI[2,3], M. FUKATA[1,3];
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Abstract: Synapse development and function require precise localization of proteins to specialized subsynaptic domains. Recent super-resolution imaging suggests a trans-synaptic nanocolumn that aligns nanometer-scale neurotransmitter release to receptors, represented by alignment between presynaptic RIM-containing and postsynaptic PSD-95-organizing nanodomains. This alignment is thought to enable precise, efficient synaptic transmission. However, it remains incompletely understood how individual pre- and postsynaptic nanodomain formation is regulated and what molecules organize nanocolumn structures although numerous trans-synaptic adhesion systems including neurexin-neuroligin and neurexin-Cbln-GluD were reported. We recently found that an epilepsy-related secreted protein, LGI1, and its receptor, ADAM22, form 2:2 heterotetrameric assembly for the trans-synaptic linkage and that LGI1 and ADAM22 regulate AMPA receptor-mediated synaptic transmission through one of MAGUKs, PSD-95. Here, we generated a mutant mouse in which the binding between ADAM22 and MAGUKs is disrupted. Blue-native gel electrophoresis revealed that this mutation greatly affected LGI1-ADAM22 supercomplex formation in the brain. Further proteomic analysis showed that ADAM22 forms protein networks with pre- and postsynaptic MAGUKs and ion-channels. We will discuss patho-physiological roles of LGI1-ADAM22-MAGUK complex that potentially links glutamate releasing sites and PSD-95/AMPA receptors to prevent epileptic seizures.

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Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.03/B76

Topic: B.06. Synaptic Transmission

Title: ErbB4 promotes inhibitory synapse formation in a manner independent of its kinase activity

Authors: *B. LUO[1], H. WANG[2], Z. DONG[3], H. L. ROBINSON[5], L. MEI[4];
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Abstract: ErbB4 is a receptor tyrosine kinase that can be activated by neuregulin 1 (NRG1). Both NRG1 and ErbB4 have been implicated as risk genes of major depression disorder and schizophrenia. The NRG1-ErbB4 signaling has been shown to be critical to the assembly of the GABAergic circuit assembly. To investigate underlying mechanisms, we co-cultured neurons with HEK293T cells expressing wild type ErbB4 and its mutants and examined their ability to

induce synapses. Remarkably, we found that a kinase dead mutant (K751M) remained able to induce synapses onto co-cultured neurons. To test this *in vivo*, we generated ErbB4-K751M mice, a knockin mutant strain that carries the single amino acid residue mutation (K751M). Unexpectedly, ErbB4-K751M mice and control mice displayed similar numbers of interneurons in the cortex and hippocampus, suggesting that interneuon migration may not require the kinase activity of ErbB4. K751M mice appeared to be normal in inhibitory synapse numbers and inhibitory postsynaptic currents. No apparent deficits were obaserved in open field, contextual fear-conditioning and pre-pulse inhibition. These results suggest that GABAergic circuit formation requires ErbB4, but not its kinase activity. We further determined whether ErbB4 may function as an adhesion molecular and identified a few transmembrane proteins that could interact with ErbB4. Experiments are underway to determine whether such interaction is involved in GABAergic circuit development. Nevertheless, our results suggest that ErbB4 might function as a cell adhesion molecule to promote synapse formation.

Disclosures: B. Luo: None. **H. Wang:** None. **Z. Dong:** None. **H.L. Robinson:** None. **L. Mei:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.04/B77

Topic: B.06. Synaptic Transmission

Support: OTKA K-18_129142
NIH/NINDS Grant NSO23945

Title: Glycinergic input to non-cholinergic neurons of the mouse basal forebrain

Authors: *I. KALLO[1,3], L. ZABORSZKY[4], M. WATANABE[5], B. PAL[6], Z. BARDÓCZI[2]; [1]Dept. of Endocrine Neurobio., [2]Lab. of Cerebral Cortex Res., Inst. Exptl. Med. - HAS, Budapest, Hungary; [3]Fac. of Information Technology, PPCU, Budapest, Hungary; [4]Rutgers The State Univ. of New Jersey, Newark, NJ; [5]Hokkaido Univ. Sch. Med., Sapporo, Japan; [6]Dept. of Physiol., Univ. of Debrecen, Fac. of Med., Debrecen, Hungary

Abstract: Cholinergic neurons receive a rich glycinergic input in all subregions of the basal forebrain (BF) extending from the medial septum rostrally through subpallidal territories towards the centromedial amygdala caudally (*Bardóczi et al, 2017*). Calcium binding protein-containing neurons in this space are in specific association with the BF cholinergic neurons by forming twisted bands along the longitudinal axis of a central dense core of cholinergic cells (*Záborszky et al, 2005*). Immunohistochemical double labelling for choline acetyl transferase (ChAT) and glycine receptor (GlyR) revealed clustered pan-GlyRα-immunoreactivity at ChAT-negative sites

in the mouse BF, indicating that non-cholinergic neurons could be also under direct influence of glycinergic neurons.
In order to investigate further the specific target cell populations of the ascending glycinergic axons from the brain stem to the BF, we have examined the non-cholinergic BF neurons for glycinergic afferents in adult male mice and analyzed them for potential interaction. Immunohistochemical double labeling was carried out to reveal, whether the glycinergic neuronal marker, glycine transporter-2-immunoreactive (GLYT2-IR) axons establish connection with BF parvalbumin (PV), calbindin (CB), and calretinin (CR)-IR neurons. These Ca-binding proteins appear in corticopetal and locally or caudally projecting GABAergic or glutamatergic neurons (*Gritti et al, 2003*) and consequently represent functionally diverse cell populations. Light- and confocal microscopic analysis of the double-labeled samples revealed GLYT2-IR axon varicosities in apposition to PV-, CB-, and CR-IR perikarya and dendrites in the nucleus of the horizontal limb of the diagonal band. Correlated electron microscopy confirmed the presence of synaptic contacts between GLYT2- and PV-, CB-, or CR-IR neurons.
These data extend the target cells population of the ascending glycinergic input of the BF and concurrently initiate efforts to distinguish further subgroups (concerning afferent- and or efferent connections, co-expressed neurotransmitters and/or receptors) within the PV-, CB- and CR-neurons to clarify the functional role of their glycinergic input.

Disclosures: I. Kallo: None. **Z. Bardóczi:** None. **L. Zaborszky:** None. **B. Pal:** None. **M. Watanabe:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.05/B78

Topic: B.06. Synaptic Transmission

Support: Swedish research council Grant 2017-03331
Wenner-Gren Foundation UPD2017-0038
Knut och Alice Wallenberg Foundation

Title: Unrevealing the role of CA10 in synapses

Authors: *L. MONTOLIU-GAYA, D. KAMINSKI, F. H. STERKY;
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Abstract: The formation and maturation of synaptic connections depend on protein-protein interactions between neuronal adhesion proteins that span the synaptic cleft to form a physical bridge between contacting neurons. Pre-synaptic neurexins (NRXNs) constitute a well-established family of such synaptic adhesion proteins, and their interactions with diverse post-

synaptic partners are thought to regulate the properties of synapses by shaping their specific molecular architecture. Mutations in the *NRXN1* gene confer genetic risk for autism and schizophrenia, but the exact function of NRXNs, their regulation and how their dysfunction can lead to disease are not well understood. The carbonic anhydrase (CA) related proteins CA10 and CA11 were recently identified as *bona fide* synaptic proteins that bind to all known NRXN isoforms. Whereas the canonical CAs play a key role in acid-base homeostasis by catalyzing the interconversion of carbon dioxide to bicarbonate, the structurally related CA8, CA10 and CA11 lack enzymatic activity due to mutations in their catalytic cores and have no known function, but are highly conserved throughout evolution. CA10 was found to bind in *cis* to conserved residues in the stalk region present in all NRXN isoforms. Unexpectedly, NRXN and CA10 have the ability to spontaneously form an intermolecular disulfide bond. This interaction leads to increased levels of specific NRXN isoforms on the cell surface of mouse and human neurons. The current work aimed to determine the *in vivo* function of CA10, and the mechanism whereby CA10 could regulate NRXNs levels. To address the functional role of CA10 at synapses, we generated mice *Ca10/11* knockouts to test the possibility of functional redundancy between the two homologous proteins using CRISPR/Cas9 methodology. Their overall viability and appearance was assessed; protein extracts from mouse brains were used to quantify the levels of different synaptic markers and; histological analysis of brain slices were performed to detect morphological alterations in the cortex and cerebellum. By understanding the role of CA10 and its interplay with such as key modulators as NRXNs, we expand our knowledge of the fundamental mechanisms that regulate the formation and function of synapses.

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Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.06/B79

Topic: B.06. Synaptic Transmission

Support: visiting fellow Berlin Institute of Health
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DFG: Ro1296/7-1, Ro1296/8-1

Title: Trans-synaptic association between Ca^{2+}-channels and AMPA receptors

Authors: *M. M. BROCKMANN[1], T. TRIMBUCH[1], T. C. SUDHOF[2], C. ROSENMUND[1]; [1]Inst. of Neurophysiol., Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Stanford Univ., Stanford, CA

Abstract: At the presynaptic terminal, incoming action potentials are efficiently transduced into vesicle fusion and neurotransmitter release. The presynaptic site of vesicle fusion is the active zone, a specialized protein network, which renders vesicles fusion competent by recruiting Ca^{2+}-channels close to docked vesicles. Recent imaging experiments have shown alignment of presynaptic active zone proteins with postsynaptic AMPA receptors, together constructing trans-synaptic nanocolumns. This suggests that presynaptic release events likely align with postsynaptic AMPA receptors physically, which in turn could enhance ligand-binding. To test how Ca^{2+}-channels and AMPA receptors are positioned at the active zone and how they align with the release sites, we combined high-pressure freezing of cultured hippocampal neurons with compatible live labeling of proteins facing the synaptic cleft. The advantage of analyzing protein localization using electron microcopy is the visualization of membrane structures and docked vesicles, respectively. We found that Ca^{2+}-channels and AMPA receptors are associated with docked vesicles whereas the trans-synaptic adhesion molecules, neurexin and neuroligin, are not. We also combined high-pressure freezing with electrical stimulation and compared Ca^{2+}-channel distribution to the docked vesicle localization before and immediately after fusion. As a result, we found a preferential release of the docked vesicles closest to Ca^{2+}-channels. Therefore, our study supports the concept of trans-synaptic functional columns in glutamatergic synapses and suggests a trans-synaptic protein network where Ca^{2+}-channels are associated with postsynaptic receptors for efficient transduction of presynaptic action potentials into postsynaptic responses.

Disclosures: M.M. Brockmann: None. **T. Trimbuch:** None. **T.C. Sudhof:** None. **C. Rosenmund:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.07/B80

Topic: B.06. Synaptic Transmission

Support: SNSF Grant PP00P3_144816
ERC Starting Grant 'SynDegrade' 679881

Title: Rapid modulation of transsynaptically aligned glutamate receptor nanocluster rings during homeostatic plasticity

Authors: *P. MUTTATHUKUNNEL[1,2], P. FREI[1], M. MUELLER[1,2];
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Abstract: Subtle changes in the organization of synaptic proteins may have profound effects on synaptic transmission and animal behavior. The *Drosophila* neuromuscular junction (NMJ) has

emerged as a powerful model system to dissect the sub-synaptic molecular architecture of presynaptic active zones using super-resolution light microscopy approaches. However, little is known about a corresponding postsynaptic organization and its relationship to presynaptic architecture. Using stimulated emission depletion microscopy, we here uncovered that postsynaptic glutamate receptors (GluRs) are organized in ring-like arrays composed of ~6 sub-diffraction GluR 'nanoclusters' at the *Drosophila* NMJ. While GluRIIA subunit-containing receptors predominantly localize to GluR nanorings, GluRIIB 'nanoclusters' can be found both, inside and outside of nanorings. Interestingly, GluR 'nanocluster' rings align with rings formed by the C-termini of the presynaptic cytomatrix protein Bruchpilot (Brp), suggesting transsynaptic co-alignment.
Genetic perturbation of the auxiliary GluR subunit neto results in less distinct receptor rings. Specifically, we detect a predominant decrease in GluRIIC fluorescence intensity outside the rings in $neto^{109}$ mutants. Moreover, we revealed rapid modulation of transsynaptically aligned Brp-GluR rings during homeostatic plasticity induced by GluR perturbation. Application of the GluR antagonist philanthotoxin-433 (PhTX) for 30 minutes results in a pronounced, scaled increase of GluRIIC fluorescence intensity within the nanoring. Moreover, PhTX treatment induces a significant increase in receptor cluster number within the ring. Interestingly, the receptor subunit GluRIIB, but not GluRIIA, undergoes rapid modulation upon PhTX incubation, resulting in increased GluRIIB fluorescence intensity and GluRIIB cluster number. Finally, we revealed a slight, but significant increase in both Brp-fluorescence intensity and Brp-cluster number without marked differences in Brp-ring diameter upon GluR perturbation. Together, our findings provide evidence for transsynaptic nanomodule rings that undergo rapid changes during synaptic plasticity.

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Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: B.06. Synaptic Transmission

Support: NIH Grant 1R01MH104319-01A1,02,03,04,05
NSF Grant 1707356
NIH Grant 2R56MH095980-06

Title: Shift in synapse structure and location advances the onset age of late-phase LTP

Authors: *H. SMITH[1], C. HAINES[2], G. CAO[2], S. L. VENTURA[2], M. H. DRAKE[2], M. KUWAJIMA[2], K. M. HARRIS[2];

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Abstract: Prior work has shown that late-phase LTP (lasting more than 3 hours) has an abrupt onset age of postnatal day 12 (P12) in *stratum radiatum* of rat hippocampal area CA1. This onset age can be advanced to P10-11 (but not P8-9) if two bouts of theta-burst stimulation (TBS) are given with a 90-minute separation (Cao and Harris 2012). In this study, we aim to discover changes in synapse structure and composition during those 90 minutes that could facilitate late-phase LTP at P10. First, we mapped through serial section electron microscopy (3DEM) the location of all synapses in unbiased sampling bricks from CA1, *stratum radiatum* at P8, P10, and P12. Preliminary results reveal synapse density doubles between P8 and P12. Most of the synapses are located on dendritic shafts at P8 and P10, while dendritic spines are the predominant location at P12. Transitional structures (rare in adults) included: surface specializations with pre and postsynaptic densities but no presynaptic vesicles, nonsynaptic protrusions, single or multi-synaptic filopodia longer than 2 microns with no heads, and lumpy or stubby protrusions often having multiple synapses. Two stimulating electrodes were positioned with a separation greater than 600 microns surrounding a recording electrode in the middle of CA1 *stratum radiatum* in three hippocampal slices from different P10 rats. Then 90 minutes after the first TBS the slices were rapidly fixed under microwave irradiation and prepared for 3DEM. Preliminary findings suggest that after TBS, there is a shift from predominantly shaft synapses to transitional and spine-like protrusions, in apparent preparation for the subsequent expression of late-phase LTP.
Cao, G. and K. M. Harris (2012). "Developmental Regulation of the Late Phase of Long-Term Potentiation (L-LTP) and Metaplasticity in Hippocampal Area CA1 of the Rat." J Neurophysiol 107(3): 902-912.

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Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.09/B82

Topic: B.06. Synaptic Transmission

Support: NIH Grant R15 NS101608-01A1

Title: The CHD protein, Kismet, is required in postsynaptic cells for presynaptic endocytosis

Authors: B. G. HARSIN, ***F. L. LIEBL**;
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Abstract: Chromatin remodeling proteins are implicated in a variety of developmental processes but their roles in neuronal synaptic function are less well understood. The chromodomain DNA-binding proteins, CHD7 and CHD8, mediate early neurodevelopmental events including neural migration and differentiation but their roles in mature synapses are relatively unexplored. Here we show that Kismet, the *Drosophila* homolog of CHD7 and CHD8, promotes endocytosis by regulating the transcription and/or protein localization of Dap160, Dynamin, and Endophilin B. Endocytosis is critical for synaptic function as it provides a mechanism to recycle membrane and synaptic proteins enabling the continued release of synaptic vesicles. Kismet is required in postsynaptic muscles of the *Drosophila* neuromuscular junction for presynaptic endocytosis. To identify the potential mechanism whereby postsynaptic Kismet regulates presynaptic endocytosis, we are examining retrograde signaling pathways including BMP signaling and signaling via neurexin-neuroligins. Kismet mutants exhibit a significant increase in the levels of Neuroligins 1 and 3 and in presynaptic BMP signaling. Although Kismet may regulate these processes independently of one another, we are currently investigating the interdepdendence of these phenotypes. A deeper understanding of how CHD proteins regulate the function of mature neurons will be required to better understand neurodevelopmental disorders.

Disclosures: B.G. Harsin: None. **F.L. Liebl:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.10/B83

Topic: B.06. Synaptic Transmission

Support: NIH Grant 1R01MH104319-01A1,02,03,04,05
NSF Grant 1707356
NIH Grant 2R56MH095980-06
NIH Grant NS102788

Title: Morphological alteration after long-term potentiation detected at the active zone of adult rat hippocampal synapses

Authors: *J. JUNG[1], J. N. BOURNE[2], L. M. KIRK[3], R. M. MARSHALL[1], K. M. HARRIS[3]; [1]Dept. of Biol., Texas A&M Univ., College Station, TX; [2]Physiol. and Biophysics, Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [3]Dept. of Neurosci. and Ctr. for learning and Memory, Univ. of Texas at Austin, Austin, TX

Abstract: Long-term potentiation (LTP) is important for learning and memory in mammalian brains and can be induced by presynaptic depolarization releasing neurotransmitters. Neurotransmitters are released by synaptic vesicles (SVs) at specialized regions called active

zones. Despite well-established mechanisms of postsynaptic receptors-mediated LTP induction, morphological changes at active zones addressing its presynaptic mechanisms remain elusive. Here we used electron tomography on hippocampal synapses of adult rat area CA1 to show that the ratio of docked SVs to the SVs within 45 nm from the presynaptic membrane (PM) at active zones significantly increases. Furthermore, docked SVs at active zones exhibited differences in terms of their contact area with the PM correlating with filaments directly linking their SVs to the PM. These relationships were altered 2 hours after LTP. Our findings suggest that the distribution of the filaments in the active zone are altered after LTP in coordination with the post-synaptic density.

Disclosures: J. Jung: None. **J.N. Bourne:** None. **L.M. Kirk:** None. **R.M. Marshall:** None. **K.M. Harris:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.11/B84

Topic: B.06. Synaptic Transmission

Support: EMBO aALTF 760-2016

Title: Alternative splicing choices for synaptic function

Authors: *A. M. GOMEZ[1], L. TRAUNMÜLLER[1], P. SCHEIFFELE[2];
[1]Univ. of Basel, Basel, Switzerland; [2]Biozentrum Univ. Basel, Basel, Switzerland

Abstract: Alternative RNA splicing has the potential to expand the coding power of the genome; yet, it is unclear how alternative splicing tunes molecular complexity for selective circuit function. We discovered that Slm2 - an RNA-binding protein - drives a highly dedicated alternative splicing program that targets to a devoted splice segment of synaptic adhesive molecules. Isoforms generated by Slm2-dependent splicing are essential for the specification of glutamatergic synapses in the hippocampus. Our data reveals that alternative splicing is a potent mechanism for neurons to generate and control the large variability observed in its synaptic interactions.

Disclosures: A.M. Gomez: None. **L. Traunmüller:** None. **P. Scheiffele:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.12/B85

Topic: B.06. Synaptic Transmission

Support: T32NS086750-05
R01MH085974

Title: Interactions between the prefrontal cortex and multiple thalamic nuclei

Authors: *D. P. COLLINS[1], A. G. CARTER[2];
[2]Ctr. for Neural Sci., [1]New York Univ., New York, NY

Abstract: Interactions between the prefrontal cortex (PFC) and thalamus are critical for cognitive function and go awry in neuropsychiatric disorders. We previously showed that mouse PFC makes reciprocal, excitatory connections with both mediodorsal (MD) and ventromedial (VM) thalamus. However, this direct excitation will be shaped by connections onto reticular thalamus (TRN) that evoke polysynaptic inhibition. Connections from the PFC arise from cortico-thalamic (CT) cells that reside in either layer 5 (L5) or layer 6 (L6). In other parts of cortex, L5 CT cells primarily project to thalamic relay nuclei, whereas L6 CT cells also project to reticular thalamus (TRN). Here we use transgenic Cre lines, conditional optogenetics and retrograde tracing to examine L6 CT output to MD, VM and TRN in the mouse brain. We first study excitatory connections onto thalamocortical (TC) cells in MD and VM. We then examine excitatory connections onto TRN cells. Lastly, we characterize inhibitory connections from TRN cells onto MD and VM. Together, our findings show how PFC provides nucleus-specific excitation and inhibition that differentially controls neural activity in multiple thalamic nuclei.

Disclosures: D.P. Collins: None. **A.G. Carter:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.13/B86

Topic: B.06. Synaptic Transmission

Support: 1F32MH105040-01

MH052804
P50AG047366
R35GM122487

Title: Synaptic neurexin-1 assembles into dynamically regulated active zone nanoclusters

Authors: *J. H. TROTTER[1], J. HAO[4], S. MAXEINER[5], T. TSETSENIS[6], Z. LIU[2], X. ZHUANG[4], T. C. SUDHOF[3];
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Abstract: Neurexins are well-characterized presynaptic cell-adhesion molecules that engage multifarious postsynaptic ligands and organize diverse synapse properties. However, the precise synaptic localization of neurexins remains enigmatic. Using super-resolution microscopy, we demonstrate that neurexin-1 forms discrete nanoclusters at excitatory synapses, revealing a novel organizational feature of synaptic architecture. Mature excitatory synapses generally contain a single nanocluster that comprises more than four neurexin-1 molecules, whereas immature synapses do not contain neurexin-1 nanoclusters. Moreover, we find that neurexin-1 is physiologically cleaved by ADAM10 similar to its ligand neuroligin-1, with ~4-6% of neurexin-1 and ~2-3% of neuroligin-1 present in adult brain as soluble ectodomain proteins. Blocking ADAM10-mediated neurexin-1 cleavage dramatically increased the synaptic neurexin-1 content, elevating the percentage of excitatory synapses containing neurexin-1 nanoclusters from 40-50% to ~80%, and doubling the number of neurexin-1 molecules per nanocluster. Taken together, our results reveal an unexpected nanodomain organization of synapses in which neurexin-1 is assembled into discrete presynaptic nanoclusters that are dynamically regulated via ectodomain cleavage.

Disclosures: **J.H. Trotter:** None. **J. Hao:** None. **S. Maxeiner:** None. **Z. Liu:** None. **T.C. Sudhof:** None. **X. Zhuang:** None. **T. Tsetsenis:** None.

Poster

037. Synaptogenesis and Activity-Dependent Development I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 037.14/B87

Topic: F.02. Behavioral Neuroendocrinology

Support: CNPq
FAPERJ
INNT

NIH
ISN
HFSP
Alzheimer's Association Canada

Title: The role of brain FNDC5/irisin in synaptic plasticity and memory in mice

Authors: *R. A. S. LIMA-FILHO[1], M. V. LOURENCO[2], O. ARANCIO[3], S. T. FERREIRA[4], F. G. DE FELICE[5];
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Abstract: Irisin is an exercise-induced myokine released upon cleavage of a precursor protein termed FNDC5, recently reported to be expressed in the hippocampus. Irisin has been reported to regulate peripheral energy metabolism and to trigger neuroprotective mechanisms. However, physiological roles of FNDC5/irisin in the brain remain poorly understood. We used lentiviral vectors harboring two different shRNA constructs targeting FNDC5 to study the role of irisin in synapse plasticity and memory. Here we show that downregulation of brain FNDC5/irisin impairs hippocampal long-term potentiation and object recognition memory, but not contextual fear memory or radial arm water maze, in C57BL/6 mice. These data support the idea that FNDC5/irisin acts in the central nervous system and impacts selective forms of memory expression and hippocampal synaptic plasticity. Thus, boosting FNDC5/irisin pathway and/or using exercise-based therapies may offer new strategies to tackle memory loss in neurodegenerative diseases.

Disclosures: R.A.S. Lima-Filho: None. **M.V. Lourenco:** None. **O. Arancio:** None. **S.T. Ferreira:** None. **F.G. De Felice:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.01/B88

Topic: B.07. Synaptic Plasticity

Title: ER stress represses neural activity and seizures through Mdm2-p53 signaling-mediated protein tranlation

Authors: *D.-C. LIU, D. EAGLEMAN, N.-P. TSAI;
Univ. of Illinois Urbana-Champaign, Uebana, IL

Abstract: Seizures can induce endoplasmic reticulum (ER) stress, and sustained ER stress contributes to neuronal death after epileptic seizures. Although inhibiting ER stress has been proposed as a way to medically reduce neuronal death after seizures, it is unclear whether and how ER stress impacts seizure onset and activity. In this study, we discovered that the acute ER stress response functions to repress neural activity through a protein translation-dependent mechanism. We found that inducing ER stress promotes the expression and distribution of murine double minute-2 (Mdm2) in the nucleus, leading to ubiquitination and down-regulation of the tumor suppressor p53. Reduction of p53 subsequently maintains protein translation, before the onset of translational repression seen during the latter phase of the ER stress response. Using an *Mdm2* conditional knockdown (cKD) mouse model, we showed that ER stress-induced p53 down-regulation, protein translation, and reduction of neural activity and seizure severity were all impaired. Importantly, these defects in *Mdm2* cKD mice were fully restored by using a p53 inhibitor, Pifithrin-α, to mimic the inactivation of p53 seen during ER stress. Altogether, our study uncovered a novel mechanism by which neurons respond to acute ER stress. Further, this mechanism appears to play a beneficial role in reducing neural activity and seizure severity. These findings caution against inhibition of ER stress as a neuroprotective strategy for seizures, epilepsies, and other pathological conditions associated with excessive neural activity.

Disclosures: D. Liu: None. **D. Eagleman:** None. **N. Tsai:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.02/B89

Topic: B.07. Synaptic Plasticity

Support: NIH grant RO1 NS12542
NSF grant EEC-1028725

Title: Excitatory single-unit responses to intracortical microstimulation in primate motor cortex suggest changes in cortico-cortical synaptic strength

Authors: *R. J. YUN[1,2,3], J. H. MISHLER[1,2,3], S. I. PERLMUTTER[4,2], R. P. RAO[5,3], E. E. FETZ[4,2,3];
[1]Bioengineering, [2]Washington Natl. Primate Res. Ctr., [3]Ctr. for Neurotechnology, [4]Physiol. and Biophysics, [5]Computer Sci. and Engin., Univ. of Washington, Seattle, WA

Abstract: Electrical stimulation via intracortical electrodes is often used to probe the cortical circuitry, but its effects have been shown to depend on parameters such as location, timing, frequency, and amplitude. We sought to better understand the consequences of intracortical microstimulation by documenting changes in the single-unit responses generated by stimulation

at a neighboring site. Macaca nemestrina monkeys were bilaterally implanted with 96-channel Utah arrays in the forearm region of primary motor cortex and trained to sit calmly at rest in a chair with both arms restrained. Single-ended single-pulse stimuli (negative leading biphasic pulse; 15µA amplitude; 200µs pulse width) were delivered to an arbitrary site (Nstim) with a random Poisson distribution at various mean frequencies of 1-300Hz. Stimulus-evoked spikes (SES) were observed in cortical sites (Nrec) up to 800µm away. SES occurred between 1.2ms and 3ms after the stimulus, with normally distributed delays (mean = 1.88±0.16ms; σ = 0.25±0.06ms; N = 47 sessions; 7 unique Nrec/Nstim pairs). The latency distribution of responses suggests mediation via a monosynaptic connection. Stimuli that generated SES also produced larger cortico-cortical evoked potentials at Nrec than stimuli that did not generate SES, further indicating activation of synaptic pathways. The probability of evoking a spike (PES) with stimulation ranged from as low as 10% to as high as 90%. Higher PES occurred with higher stimulus intensities and closer proximity of the Nrec and Nstim sites. Higher PES also occurred with stimuli delivered at intervals similar to the neuron's preferred inter-spike interval during spontaneous activity, calculated by the peak of the spike's autocorrelation. Tonic stimulation at a constant rate altered these probabilities within as quickly as 30 seconds, monotonically changing the PES to an asymptotic limit dependent on the Nrec/Nstim pair. High-frequency stimulation (>20Hz) caused a reduction in the PES (N=4) and low frequency stimulation (<10Hz) caused an increase in PES (N=2); intermediate frequencies did not have consistent consequences. These changes did not persist after the termination of stimulation; PES returned immediately to baseline. The induced effects on PES suggest short-term strengthening or weakening of synaptic connections from Nstim to Nrec depending on the frequency of stimulation. Overall, these results show how parameters of intracortical microstimulation affect local cortical circuitry and provide insights relevant to optimizing stimulation protocols for cortical plasticity paradigms.

Disclosures: R.J. Yun: None. **J.H. Mishler:** None. **S.I. Perlmutter:** None. **R.P. Rao:** None. **E.E. Fetz:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.03/B90

Topic: B.07. Synaptic Plasticity

Support: Focus Program Translational Neuroscience (FTN) scholarship

Title: Kinetics of short term plasticity differ between GABAergic and glutamatergic systems in layer II/III of the mouse barrel cortex

Authors: *A. LOMBARDI, H. J. LUHMANN, W. KILB;
Inst. for Physiol., Johannes Gutenberg Univ. Mainz, Mainz, Germany

Abstract: Short-term plasticity (STP) is a widespread mechanism underlying sensory plasticity in cortical networks. Differential STP between excitatory and inhibitory systems plays an important role for processing complex physiological sensory inputs. To investigate how STP influences the processing of information in layer ll/lll of the barrel cortex, a prototypical sensory cortical area with well defined projections, we performed whole-cell patch-clamp experiments on visually identified pyramidal cells in acute slices and combined electrical stimulation with optogenetic activation of PV-interneurons via Channelrhodopsin-2 (ChR2). These experiments revealed that 10 electrical stimuli delivered at 5 Hz reduce the amplitude of glutamatergic responses by 32±4.2% (n=8). This short term depression (STD) lasted 5 s. This electrical stimulus also reduced the amplitude of GABAergic responses by 54±3.3% (n=8), which recovered within 10 s. Optogenetic stimulation of PV-positive GABA terminals with a similar burst protocol reduced the amplitude of GABAergic responses by 45±3.5% (n=9), which recovered after 20 s. Finally, isolated optogenetic burst stimulation of GABAergic terminals reduced the amplitude of electrically stimulated glutamatergic responses by 25±7.6% (n=7). This STD had a delayed onset and was maximal about 2 s after the optogenetic burst, suggesting that activation of extrasynaptic receptors by spillover contributed to this effect. In summary, these results indicate that in layer ll/lll GABA terminals show a stronger STD than glutamatergic synapses and that activation of extrasynaptic $GABA_A$ receptors may contribute to STD at high frequency stimulation. The differential STD between GABAergic and glutamatergic inputs is probably important for gating information flow, while the additional delayed STP can maintain the global excitation/inhibition ratio during strong sensory stimulation.

Disclosures: A. Lombardi: None. **H.J. Luhmann:** None. **W. Kilb:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.04/B91

Topic: B.07. Synaptic Plasticity

Title: Processing of spatial information enhanced by non spatial information in hippocampal granule cells

Authors: *N. NAKAJIMA, G. TASAKA, H. HAYAKAWA, T. AIHARA;
Tamagawa Univ., Tokyo, Japan

Abstract: Spatial information (place) and non-spatial information (chiefly odor) are integrated in hippocampal dentate granule cells (GCs). GCs was independently received two inputs from entorhinal cortex. It was reported that spatial information is propagated at theta oscillation (4-8 Hz) to the medial dendrite (MD) of GCs. On the other hands, non-spatial information is propagated at gamma oscillation (20-40 Hz, average 21.0 Hz) to the lateral dendrite (LD) of

GCs. However, how those information is integrated is still unknown.
To investigate the integration mechanism of two inputs to MD and LD, the frequency responses of MD and LD of GCs were measured using rat hippocampal slices. We applied 5 pulses stimulus at 10-40 Hz to MPP or LPP. During experiment, to prevent the synaptic plasticity by frequency inputs from inducing, D-APV, NMDA-receptor antagonist was applied. In addition, GABAA receptor antagonist, picrotoxin, was applied. As the physiological experimental result, responses were transiently decreased at MD of GCs in the both cases, with and without inhibitory inputs. On the other hand, successive responses for five inputs were sustained at DD of GCs. However, when inhibitory inputs were blocked by picrotoxin, those responses were decreased at DD of GCs. The result suggest that inhibitory inputs may stabilize responses at the DD of GCs.
In addition, computer simulation was performed using a multi-compartment model of the GC by using NUERO simulator. This model was fixed by parameter fitting for the physiological data. Theta burst input and random pulses (10-40 Hz, gamma) were applied to MD and LD of GC, respectively. As the computational experimental result, the temporal-pattern sensitivity for burst inputs was not clear when theta burst inputs applied to MD. However, when random pulse inputs were simultaneously applied to LD at 10 Hz, GC activation for theta burst input was increased. Moreover, 20Hz-30Hz random pulse inputs were simultaneously applied to LD, GC activation was more facilitated so that temporal pattern sensitivity for theta input was clearly observed. Our results suggest that processing of spatial information was enhanced depending on the input frequency of non-spatial information in GC.

Disclosures: **N. Nakajima:** None. **G. Tasaka:** None. **H. Hayakawa:** None. **T. Aihara:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.05/B92

Topic: B.07. Synaptic Plasticity

Support: SERB-DST grant PDF/2017/001803

Title: Balancing information transmission and energy use at unreliable hippocampal synapses

Authors: ***G. MAHAJAN**, S. NADKARNI;
Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: Synaptic transmission consumes a significant proportion of energy during neural activity in the mammalian brain. In this context, one may ask if the unreliable nature (i.e., low release probability) of chemical synapses is a feature (helping reduce energy use), or a bug (failing to transmit action potentials). A proper interpretation of the functional role of synaptic

failures requires also taking into account the accompanying activity-dependent changes in neurotransmitter release that occur on a time scale of milliseconds to seconds. How unreliability and short-term plasticity work together to shape the energy efficiency of synaptic information transmission remains an important but not completely understood question. We investigate this trade-off between information and energy use at excitatory CA3 to CA1 connections in the hippocampus, which typically have low basal release probabilities (mean ~ 0.2) and exhibit short-term facilitation (STF). Activity of CA3 place cells is known to encode the animal's spatial location as short, variable high-frequency spike bursts, which provides a well-defined context to define a notion of information. Using a physiologically realistic model of synaptic vesicle release dynamics, we characterize how the basal release probability (Pv) and facilitation regulate the transmission of natural spike trains at individual synapses. Our analysis highlights the role of facilitation and vesicle pool size in enhancing the fidelity of low Pv synapses, and indicates that physiologically observed STF (which varies inversely with Pv) makes information transmission independent of Pv. Further, our simulations suggest that STF at CA3-CA1 synapses is tuned to maximize information transmission in an efficient manner. Broadly, our results suggest that study of a synapse in the context of competing resource and functional constraints may provide unique insights into synaptic design across brain areas.

Disclosures: **G. Mahajan:** None. **S. Nadkarni:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.06/B93

Topic: B.07. Synaptic Plasticity

Support: NSF-RTG 1547394

Title: Roles of synaptic plasticity in a reduced CA1 model

Authors: ***D. POLL**[1], D. A. DOMBECK[2], W. L. KATH[1];
[1]Applied Mathematics, [2]Neurobio., Northwestern Univ., Evanston, IL

Abstract: Recent advancements in two-photon imaging with fluorescent glutamate reporters have allowed experimentalists to probe the pattern of pre-synaptic inputs on CA1 basal dendritic branches. Of particular interest for investigation is the possibility of spatial and/or temporal clustering of inputs on dendritic branches within a few microns and/or milliseconds of one another.
We present a reduced dendritic model that explores various spike-timing-dependent plasticity (STDP) rules and the parameter spaces in which clustered synaptic input could form. We simulate a mouse running through multiple one-dimensional environments over multiple trials,

with ensembles of cells in CA3 producing correlated inputs onto CA1 dendrites. We use this model to investigate the possibility of the correlated input leading to synaptic clustering, and the extent to which this clustering depends upon the plasticity model and the associated parameters. One expects, for example, that the calcium diffusion lengthscale and the correlation time of upstream inputs play key roles in the development of localized clustering on dendritic branches.

Disclosures: D. Poll: None. **D.A. Dombeck:** None. **W.L. Kath:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.07/B94

Topic: B.07. Synaptic Plasticity

Support: R01 NS108778
R01 NS108778-01S1

Title: Fasting effects in synaptic transmission depend on the basal feeding

Authors: *G. MACIAS-MENDEZ[1,2], R. A. JORQUERA[3,4];
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Abstract: Neuronal transmission is highly plastic and undergoes short-term changes in synaptic efficacy during high-frequency nerve-activity episodes. After the episode ceases, these changes are restored to their initial values over time, a phenomenon known as short-term synaptic memory (STSM). STSM is key for learning and working memory, demanding a high level of energy. Interestingly, starvation induces behavioral changes including memory and synaptic computation impairments; which is associated with energy state modifications. Nevertheless, the effect of starvation on STSM remains elusive. To understand the effects of starvation in neuronal communication, we scrutinized the effect of acute fasting in synaptic transmission and STSM at the *Drosophila* glutamatergic synapses. Control strains after starving display nerve-evoked hyperexcitability and progressive nerve fatigue, a condition prevented in flies grown in supplemented food. Fasting in well-fed animals, impairs STSM in two quantal release probabilities and increases the asynchronous neurotransmission at rest. Also, high-frequency nerve-activity induces a depletion in the asynchronous release fraction which recovers at low-frequency. This type of plasticity is unobserved in starved animals, suggesting a vesicular trafficking defect. Our work shows a novel type of short-term plasticity modulating the asynchronous release. Also, we show that starvation induces alteration in synaptic transmission and STSM in well-fed flies. Our observations are consistent with energy reduction after

starvation in the normal-fed animals but are inconsistent for flies grown in supplemented food; as neurotransmission and recycling persist with nerve activity without increasing miniature events. During starvation, autophagy links membrane recycling via the endo/lysosomal system; thus, an impairment in vesicle trafficking and reformation may account for STSM deficit. However, calcium leakage during lysosome activity may account for the basal changes in the asynchronous release and spontaneous activity after starvation. A molecular screening required to identify the molecular regulation of these types of plasticity, and their importance to understanding many neurological conditions modulated with diet and starvation is discussed.

Disclosures: **G. Macias-Mendez:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH. **R.A. Jorquera:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.08/B95

Topic: B.07. Synaptic Plasticity

Support: NIH GM103554
UNR VPRI

Title: Viral expression of a MIRO-binding domain peptide depletes mitochondria from the calyx of Held presynaptic terminal

Authors: M. SINGH[1], H. DENNY[2], ***R. B. RENDEN**[3];
[1]Physiol. and Cell Biol., Univ. of Nevada, Reno, NV; [3]Physiol. and Cell Biol., [2]Univ. of Nevada Sch. of Med., Reno, NV

Abstract: Mitochondria are trafficked form the cell body to sites of activity on microtubules, mediated by kinesin motors. Adaptor proteins TRAK/Milton and mitochondrial Rho-GEF (MIRO) link mitochondrial cargo to these motors. Presynaptically localized mitochondria buffer $Ca^{2+}$and generate ATP to support neurotransmission. Impaired mitochondrial trafficking occurs in many neurodegenerative diseases, but the effect of mitochondrial localization*per se*on neurotransmission is unclear. The goal of this study was to mislocalize mitochondria from the presynaptic terminal but leave mitochondrial function intact. Previous studies have shown that expression of the MIRO-binding domain of TRAK (MBD) sabotages anterograde trafficking of mitochondria in neuronal cultures. In an attempt to deplete the calyx of Held presynaptic terminal of mitochondria, we expressed MBD via AAV stereotaxically injected into the ventral cochlear nucleus of 0-1 day old mice, selectively expressing this peptide in the neurons that form the calyx of Held. Within three weeks, high resolution confocal reconstruction of the calyx

terminal showed that >60% of mitochondrial content was lost. The effect of this loss on transmission was examined using Ca^{2+}imaging and electrophysiology.To our surprise, synaptic transmission was not dramatically altered in terminals expressing MBD. Short stimulation trains (100-300 Hz, 500 ms) showed no appreciable changes relative to WT controls. When long stimulation trains (100 Hz, 2 min) were applied, transmission was not only maintained, but was slightly facilitated relative to controls. This result differs from inhibition of mitochondrial ATP production, which substantially impaired sustained transmission. Cytosolic Ca^{2+}load was increased in MBD-expressing terminals. This phenotype was confirmed using both genetically encoded indicators (jRGECO1a), and also with a synthetic reporter (Fura Red) where ATP levels could be maintained. Due to tight Ca^{2+}-SV coupling at the mature calyx of Held, we presume that this increased cytosolic Ca^{2+}load increased mobilization of the recycling pool prematurely, leading to the observed facilitation. Additional experiments are underway to determine if glycolysis upregulation is utilized to compensate for loss of presynaptic mitochondria. We will track mitochondrial trafficking in the ventral stria axons that lead to the calyx. Older animals will also be examined, to see if mitochondria can be further depleted from the presynaptic terminal.

Disclosures: **R.B. Renden:** None. **M. Singh:** None. **H. Denny:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.09/B96

Topic: B.06. Synaptic Transmission

Title: Removal of calcium dependent regulation of ATP binding of Syn I has distinct effects at excitatory and inhibitory synapses

Authors: *M. MOSCHETTA[1], A. DE FUSCO[2], S. SACCHETTI[3], G. LIGNANI[4], M. ORLANDO[5], P. BALDELLI[6], F. BENFENATI[7];
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Abstract: Synapsin I (SynI) is a phosphoprotein that regulates synaptic vesicle (SV) dynamics at the presynaptic terminal. Nonsense and missense mutations in human SYN1 gene are related to several diseases such as epilepsy and autism spectrum disorder and SynI knockout (KO) mice are epileptic. SynI binds ATP in a Ca^{2+}-dependent manner thanks to the coordination of a glutamate residue (E373). As ATP binding regulates SynI oligomerization and SV clustering, we analyzed the effect of E373K mutation on neurotransmitter release and short-term plasticity in excitatory

and inhibitory synapses. We coupled electrophysiology (patch-clamp recordings) with electron microscopy in primary SynI KO hippocampal neurons in which either the human wild type or the E373K mutant SynI were re-introduced by infection with lentiviral vectors. Our data showed an increase in the frequency of miniature postsynaptic currents (mPSCs), without changes in the amplitude in both excitatory and inhibitory neurons expressing E373K-SynI. Excitatory E373K-Syn I neurons showed reduced evoked EPSC amplitude attributable to a reduction of the readily releasable pool (RRP) while, on the contrary, inhibitory E373K-Syn I neurons showed enhanced evoked IPSC amplitude and RRP size. While no effects in the dynamics and steady state of depression were detected, both excitatory and inhibitory E373K-Syn I neurons failed to recover after stimulation with long high-frequency trains. No mutation-induced changes were observed in network firing/bursting activity as determined with multi-electrode extracellular recordings. Our data suggest that the Ca^{2+}-dependent regulation of ATP-binding to SynI plays important roles in spontaneous and evoked neurotransmitter release that differentially affect the strength of excitatory and inhibitory transmission.

Disclosures: M. Moschetta: None. **A. De Fusco:** None. **S. Sacchetti:** None. **G. Lignani:** None. **M. Orlando:** None. **P. Baldelli:** None. **F. Benfenati:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.10/B97

Topic: B.07. Synaptic Plasticity

Title: Synapsin null alters multiple neurotransmitter release functions at *Drosophila* NMJ

Authors: *A. GONZÁLEZ RUIZ[1], R. A. JORQUERA[3], P. FELICIANO[4], J. GUZMAN-GUTIÉRREZ[2];
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Abstract: The availability of synaptic vesicles (SVs) and their timing for fusion are critical for neurotransmitter release and neural communication. Synapsins (Syn) are abundant phosphoproteins associated reversibly with SVs and cytoskeleton. Syn is highly conserved in the animal kingdom and is thought to regulate SVs trafficking and short-term plasticity. Syn function compromises learning and memory. However, its role in synaptic transmission is still under investigation. Here we analyzed Syn null by recordings of synaptic transmission under voltage-clamp at the *Drosophila* glutamatergic model. We found that Syn null increases the probability of SV fusion by decreasing the sensitivity to calcium. Also, Syn null increased asynchronous release, alters short-term plasticity and synaptic memory. A use-dependent model for

neurotransmission induced by high-frequency nerve activity and masked plasticity under depression is discussed.

Disclosures: A. González Ruiz: None. **R.A. Jorquera:** None. **P. Feliciano:** None. **J. Guzman-Gutiérrez:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.11/B98

Topic: B.06. Synaptic Transmission

Support: NIH NS111749
NIH MH084874

Title: Group II and III metabotropic glutamate receptors signal via the Gβγ-SNARE pathway

Authors: *C. E. DELBOVE[1], Z. ZURAWSKI[3,1], R. M. LAZARENKO[4], H. E. HAMM[5], S. T. ALFORD[2];
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Abstract: Inhibitory $G_{i/o}$-coupled G-protein coupled receptors in the presynaptic terminal can inhibit exocytosis via multiple mechanisms. The most well-studied mechanism is the inhibition of calcium influx into the presynaptic terminal by G protein βγ subunits binding to voltage-gated calcium channels. Inhibition of exocytosis downstream of calcium entry can also occur via Gβγ binding to the SNARE complex and occupying the binding sites of the fusogenic calcium sensor synaptotagmin I. The SNAP25Δ3 mouse model, which carries a mutation truncating the SNAP25 protein by the C-terminal three residues, is deficient in the Gβγ-SNARE pathway, and permits investigators to distinguish between the two pathways. It was previously shown that the inhibitory action of 5-HT_{1B}, which is known to signal via Gβγ-SNARE, is disrupted in this model, while $GABA_B$, which is thought to signal via inhibiting calcium influxes, is not. Here, in Schaffer collateral to CA1 synapses, we show that the inhibitory effect of the group II and group III metabotropic glutamate receptors on excitatory field potentials is selectively diminished in SNAP25Δ3. In paired recordings of Schaffer collateral to CA1 synapses, the mGluR2/mGluR3 agonist eglumegad inhibited EPSCs in a concentration-dependent manner in slices from wild-type animals, but had the opposite effect in SNAP25Δ3 slices. Eglumegad also increased the paired pulse ratio in WT slices and slightly decreased it in in SNAP25Δ3 slices. The differential response to eglumegad demonstrates that Gβγ-SNARE binding strongly contributes to mGluR2/mGluR3-mediated presynaptic inhibition independently of Gβγ binding to VGCCs. In

tandem, these two Gβγ-dependent mechanisms can provide more precise control over synaptic vesicle release and neurotransmission that may be exploited therapeutically. Group II mGluR agonists including eglumegad are being investigated as potential treatments for traditionally difficult to treat conditions including schizophrenia and chronic pain.

Disclosures: C.E. Delbove: None. **Z. Zurawski:** None. **R.M. Lazarenko:** None. **H.E. Hamm:** None. **S.T. Alford:** None.

Poster

038. Short-Term Plasticity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 038.12/B99

Topic: B.06. Synaptic Transmission

Support: NIH MH084874
NIH MH101672

Title: Gβγ exhibits isoform-dependent ability to inhibit the activity of fusogenic C2AB-domain containing calcium sensors

Authors: Z. ZURAWSKI[1], C. DELBOVE[1], *S. T. ALFORD[2];
[1]Anat. and Cell Biol., [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Presynaptic Gi/o-coupled GPCRs activate Gβγ subunits, which can inhibit exocytosis through one of two known pathways: inhibition of calcium fluxes through binding to voltage-gated Ca^{2+} channels, or downstream of Ca^{2+} channels by binding directly to the SNARE protein complex and displacing the Ca^{2+} sensor synaptotagmin I. During repetitive stimulation, the activity of Gi/o-coupled GPCRs such as 5-HT_{1B}, which signal via the Gβγ-SNARE mechanism, is most pronounced at the first stimulation and diminishes with subsequent stimulations in a Ca^{2+}-dependent manner. In contrast, the activity of Gi/o-coupled GPCRs which signal via voltage-gated Ca^{2+} channels, such as $GABA_B$, remains constant. Because Ca^{2+} accumulation is thought to not alter the fusogenic capacity of syt I, we propose that this differential GPCR activity is due to the subsequent activation of another synaptotagmin isoform. Two candidates are syt VII, which interfaces with the SNARE complex, but which we show is insensitive to the competitive effect of Gβγ, for binding to the SNARE complex and in evoking fusion in a reduced fusion assay, and Doc2, a fusogenic calcium sensor that binds SNAREs, and like syt I, is sensitive to Gβγ. Here we show that genetic ablation of syt VII does not enhance 5-HT_{1B} receptor-mediated inhibition during stimulus trains, while we confirm that it reduces synaptic enhancement during repetitive stimulation. As for responses in wild-type recordings, responses late in short stimulus trains are not inhibited by 5-HT_{1B} receptor activation, while those at the start of the train are. While we show that fusogenic C2AB Ca^{2+} sensors exhibit isoform-dependent ability to be competitively

displaced from the SNARE complex by Gβγ, the molecular basis for this specificity remains unclear. The loss of Gβγ SNARE-mediated inhibition during repetitive stimulation is, however, Ca^{2+} sensitive. It is possible that Ca^{2+} accumulation during stimulus trains displaces high Ca^{2+} affinity sensors such as Doc2, but it is also remains possible that syt I-SNARE interactions require lower Ca^{2+} concentrations than is required for syt I's fusogenic effect.

Disclosures: Z. Zurawski: None. **C. Delbove:** None. **S.T. Alford:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.01/B100

Topic: B.07. Synaptic Plasticity

Support: VALEAS (Rome)

Title: From gut to brain function: Gain in gut bifidobacteria alters $GABA_A$ subunits expression and enhances hippocampal plasticity in adult male rats

Authors: *F. BIGGIO[1], M. C. MOSTALLINO[2], G. TALANI[2], V. LOCCI[3], L. BOI[1], R. MOSTALLINO[1], C. PORCEDDA[1], E. SANNA[1,2], G. BIGGIO[1,2];
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Abstract: Increasing evidence strengthens the crucial role of gut microbiota (GM) as a powerful regulator of brain physiology and behaviour in rodents and humans. Ingestion of specific live bacteria (probiotics) therefore appears to be a potential treatment for several neurological disorders. Administration of adequate and specific probiotics may confer, in animals, a benefit for health affecting behaviour and several brain functions. A large plethora of studies suggested the hippocampus as a possible target for this fine tuning. Recent evidences highlighted that structural integrity of the hippocampal formation is contingent on the presence of a healthy GM. Nevertheless, the mechanism involved in the ameliorating action of GM on brain, behavioral and cognitive functions, has not yet fully understood. It has been recently proposed that changes in GM alter the stress responses to the hypothalamic-pituitary-surrenal (HPA) axis, an effect that may involve the GABA inhibitory system, one of the first candidates in the modulation of emotions. Starting from these evidences, here we studied in adult male rats the long-lasting effect of a 1-2 months chronic treatment with a preparation (TRIBIF) of three different Bifidobacteria (*Longum, Breve, Infantis*) on GABAergic system and hippocampal plasticity as well as HPA axis responsiveness to acute stress in adult naïve rats measuring the plasma levels of hormones such as allopregnanolone (AP) and corticosterone (CTS). TRIBIF treatment induced a decrease in

basal plasmatic content of AP with no changes in CTS amount. Furthermore, the treatment failed to change foot-shock-induced increase of CTS levels when compared to vehicle group. Western blot analysis showed that two months of TRIBIF treatment reduced the expression of α1, α3, α4, α5 and δ $GABA_AR$ subunits while increased γ2 subunit. Patch-clamp experiments performed in dentate gyrus granule cells (DGGC) showed no change in GABA-mediated synaptic currents whereas significantly decreased the tonic component of GABAergic inhibition. The lack of TRIBIF towards the response to an acute stress worth to be further investigated given that treatment was carried out in healthy animals suggesting that beneficial effects of TRIBIF could well manifest themselves in organisms with an altered GM. Our results, together with recent findings show the potential effect of probiotics ingestion and the possible role in the treatment of psychiatric disorders such as depression. Further supports are necessary to understand the crucial role of the GM on the synaptic plasticity and brain function. Founded by VALEAS.

Disclosures: **F. Biggio:** None. **M.C. Mostallino:** None. **G. Talani:** None. **V. Locci:** None. **L. Boi:** None. **R. Mostallino:** None. **C. Porcedda:** None. **E. Sanna:** None. **G. Biggio:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.02/B101

Topic: B.07. Synaptic Plasticity

Support: NSFC#31500842

Title: Physical exercise modulates cortical neural plasticity to enhance learning and memory functions

Authors: *L. ZHANG, K. CHEN, J.-A. WEI, K.-F. SO;
Jinan Univ., Guangzhou, China

Abstract: Physical exercise training has well-known effects on the improvement of cognitive functions and mental status. The neurobiological mechanism, however, is still poorly understood. Current knowledge mainly focuses on the facilitation of hippocampal neurogenesis, or neuroprotection against neurotoxicity by exercise. On the other hands, we know little about the dynamic change of dendritic spines, which form the structural basis of neural plasticity and learning memory. Our group generated mouse chronic restraint stress models and found excess pruning of cortical spines by *in vivo* 2-photon transcranial imaging, in association with deficits of sensory dependent working memory. The adoption of treadmill exercise training effectively recued spine pruning and recovered memory deficits. Further molecular studies suggested elevation of brain derived neurotrophic factor (BDNF) in exercised brain. At the downstream of BDNF, treadmill training persistently activates mechanistic target of rapamycin (mTOR)

signaling pathway, which helps to facilitate the expression of synaptic proteins. Moreover, exercise training increases spine formation rate in cortical regions and potentiated calcium spikes to improve synaptic plasticity, thus contributing to better acquisition of motor skill memory. Using pharmacological inhibition, we demonstrated that mTOR activation is necessary for exercise-improved neural plasticity. Those results enrich our understandings for environmental influences on neural plasticity, and further support the intervention of psychiatric disorders or cognitive dysfunctions using exercise paradigms.

Disclosures: **L. Zhang:** None. **K. Chen:** None. **J. Wei:** None. **K. So:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.03/B102

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R15NS099983

Title: Estrogen signaling is required for treadmill exercise mediated effects on synaptic plasticity around axotomized spinal motoneurons

Authors: *J. C. WILHELM, A. A. BRUCE, G. DICKINSON, V. L. KENNEDY, S. T. WILSON;
Psychology, Col. of Charleston, Charleston, SC

Abstract: After peripheral nerve transection, changes in spinal cord circuitry occur including the withdrawal of synaptic inputs from the somata and proximal dendrites of axotomized spinal motoneurons. Moderate daily treadmill exercise after transection injuries has been shown to mitigate the reduction in synaptic coverage; however, different exercise protocols are required in male and female animals. The mechanisms that underlie the sex-dependent effect of exercise are not well understood; however, androgen receptor signaling has been shown to be an important factor in this process. In this study we tested the hypothesis that estrogen receptor (ER) signaling also is a part of the mechanism by which treadmill exercise mediated its effects on synaptic inputs. The lateral gastrocnemius motorneuron pools were retrogradely labeled in both sides of the spinal cord of gonadally intact male and female C57BL/6 mice. One week later, the sciatic nerve was transected unilaterally mid-thigh. Mice then were treated with various combinations of estradiol (E) treatment, ER antagonist treatment, and treadmill exercise during the two weeks. Fourteen days after the nerve transection surgery, the average synaptic coverage of glutamatergic and GABAergic inputs onto labeled motoneurons was assessed. We found no significant reduction in coverage in mice treated with E alone. Additionally, we found that blocking ER signaling during treadmill exercise prevented the sustaining effects of the exercise and resulted

in an increased loss of inputs compared to mice not receiving the ER antagonist. Based on these results we suggest that ER signaling is an important part of the machinery required for the sex-dependent exercise-mediated effects on synaptic inputs onto axotomized motoneurons after sciatic nerve transection.

Disclosures: J.C. Wilhelm: None. **A.A. Bruce:** None. **G. Dickinson:** None. **V.L. Kennedy:** None. **S.T. Wilson:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.04/C1

Topic: B.07. Synaptic Plasticity

Support: KAKENHI 17J04137
KAKENHI 18K14844
KAKENHI 19H03336
KAKENHI 17H06311

Title: Circuit remodeling in the motor cortex during motor learning

Authors: *J. SOHN[1,2], Y. KUBOTA[1,3], Y. KAWAGUCHI[1,3];
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Abstract: For skillful movement, the neuronal circuitry in the motor cortex should be optimally organized. Repetitive training for a novel motor skill induces a reorganization of neuronal wiring in the motor cortex involving synaptogenesis, indicated by spine formation on pyramidal cell dendrites. However, the origin of synaptic inputs to those spines formed during motor learning has remained to be clarified. In order to depict the circuit remodeling in the motor cortex, here we characterized the presynaptic axon terminals innervating the newly-formed spines during motor learning. To observe spine dynamics in vivo under a two-photon microscope, we used Thy1-eGFP-M mouse line, in which green fluorescent protein is expressed in layer 5 pyramidal cells. To quantify motor learning, we applied success rate of single-seed reaching task (Xu et al., Nature, 2009). The spine formation rate at the dendritic tuft in layer 1 was significantly correlated with the success rate increase after the reaching training, indicating that the spine formation reflects the refining of the motor skill. To identify whether the presynaptic axon comes from the cortex or thalamus, we fixed the brains immediately after the two-photon microscopy, followed by immunohistochemistry for the excitatory presynaptic type markers (type I and II vesicular glutamate transporters), simultaneously with an excitatory postsynaptic marker.

Confocal laser scanning microscopy at a synaptic resolution revealed that the new spines formed during motor learning were frequently innervated by corticocortical axon fibers. The dynamic corticocortical rewiring indicates that the corticocortical network is crucial for acquisition of a novel motor skill.

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

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.05/C2

Topic: B.07. Synaptic Plasticity

Support: HHMI Gilliam Fellowship
NSF GRFP
NIH Grant EY02858
Mathers Foundation
NIH Grant NS091144

Title: Increased learning-induced spine stability predicts motor skill performance

Authors: *E. ALBARRAN[1], A. J. RAISSI[2], C. J. SHATZ[3], J. B. DING[4];
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Abstract: Dendritic spine dynamics of Layer 5 Pyramidal neurons (L5PNs) are thought to be physical substrates for motor learning and memory of motor skills. Here we explore this idea by studying mice lacking Paired immunoglobulin receptor B (PirBKO). PirB is expressed in pyramidal neurons throughout the forebrain, and regulates activity dependent synaptic plasticity in the hippocampus and visual system (Djurisic et al, Mol Psychiatry 2018). In motor cortex of PirBKO mice, there is an increase in spine density on apical dendritic tufts of L5PNs, as well as an increase in mEPSC frequency in whole cell recordings from neurons in acute slice, compared to WT littermate controls. To investigate the elevated spine density further, chronic 2- photon imaging of PirBKO;Thy1-YFP mice was conducted. PirBKO mice have elevated rates of spine formation and to a lesser degree, spine elimination, yielding a net increase in spine density compared to WT. Given these changes in spine dynamics, we hypothesized that PirBKO mice would exhibit aberrant motor learning behavior. Surprisingly, adult PirBKO mice learned a single-pellet reaching task significantly faster than littermate controls. Chronic imaging of L5PN dendrites throughout the learning period revealed that PirBKO mice exhibit significantly elevated spine formation rates during the early learning stage but spine elimination rates remain

unchanged throughout. Moreover, newly formed spines in PirBKOs survive for longer periods compared to controls. Magnitudes of learning-induced spine formation and stability of newly formed spines in motor cortex correlate with task performance, suggesting that such specific structural changes may be advantageous and can translate into enhanced acquisition and maintenance of motor skills.

Disclosures: E. Albarran: None. **A.J. Raissi:** None. **C.J. Shatz:** None. **J.B. Ding:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.06/C3

Topic: B.07. Synaptic Plasticity

Support: ERC Advanced Investigator Grant (322744; LO)
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Wings for Life
Karolinska Institutet Research Foundations (LO)
Karolinska DPA (LO)

Title: Modulation of activity-driven NgR1 regulation by dopamine and serotonin

Authors: *A. T. BRODIN, G. A. ZISIADIS, K. WELLFELT, L. OLSON, T. E. KARLSSON; Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Nogo receptor 1 (NgR1) is an important receptor for several myelin associated inhibitors of neurite growth. By binding these ligands, which despite their name are also expressed by neurons, NgR1 restricts plasticity during development, learning and injury. We have previously shown that NgR1 can be rapidly downregulated by NMDA stimulation, likely to allow for windows of plasticity after neural activity. Dopamine and serotonin are known to modulate plasticity and memory formation and are proposed to act as significance signals determining what experiences are important to encode. Here we use primary hippocampal cell cultures to investigate whether dopamine and serotonin can modulate the NMDA-driven regulation of NgR1. We also report on the concentration dependence of NMDA-driven regulation of NgR1, with high concentrations of NMDA having the opposite effect of low concentrations.

Disclosures: A.T. Brodin: None. **G.A. Zisiadis:** None. **K. Wellfelt:** None. **L. Olson:** None. **T.E. Karlsson:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.07/C4

Topic: B.07. Synaptic Plasticity

Support: MH071533 (RAS)
T32-DC011499 (EMP)

Title: Sex differences in dendritic spine density and morphology in mouse auditory and visual brain regions in adolescence and adulthood

Authors: *E. M. PARKER[1], N. L. KINDJA[2], C. E. CHEETHAM[3], R. A. SWEET[1];
[1]Dept. of Psychiatry, [3]Dept. of Neurobio., [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Synapse remodeling is required for circuit refinement during adolescence and results in reduced dendritic spine density (DSD) in adulthood. We demonstrated previously that DSD decreases from P28 to P84 in primary auditory cortex layer 2/3 in male mice. We recently surveyed dendritic spines in male and female mice in primary and secondary auditory and visual regions to identify potential sex differences and better understand synaptic remodeling in adolescence, hypothesizing DSD is reduced from P28 to P84 in both sexes. C57Bl/6J mice were exposed to AAV via bulk regional viral injection on P0-2. Brains were extracted on P28 or P84 (n=15). 3D image stacks of randomly selected secondary basilar dendritic segments were captured and assessed using quantitative confocal microscopy. Dendritic protrusions from 287 segments (97 neurons) were counted and assigned to 1 of 8 types: short stubby, long stubby, short mushroom, long mushroom, long thin, branched, atypical dendritic spines or filopodia. Age did not significantly affect DSD. DSD was significantly decreased in females ($f=8.279$, $p=0.005$), with no age*sex interaction. Layer significantly impacted DSD after adjusting for multiple comparisons and the laminar pattern of DSD: layer 2/3>4=5/6 was preserved across ages and sexes (layer 2/3 and 4 $p=0.002$; layer 2/3 and 5/6 $p<0.000$), with no significant age*layer nor sex*layer interactions. Long mushroom spine density was significantly decreased from P28 to P84 ($f=4.889$, $df=1$, $p=0.029$). Short stubby ($f=13.153$, $p<0.000$), long stubby ($f=12.512$, $p=0.001$) and short mushroom ($f=4.573$, $p=0.035$) spine density were significantly reduced in females. We demonstrate for the first time that DSD is significantly reduced in females in auditory-visual sensory cortex and propose the decrease is driven by fewer short stubby, long stubby and short mushroom spines. Finally, although age did not significantly affect DSD in our model, long mushroom spine density was significantly reduced from P28 to P84, evidence of synapse remodeling during adolescence. These data will serve to inform our understanding of sex differences in synapse compositions over neurodevelopment in higher

mammals and future studies investigating spine alterations in animal models of neurological or psychiatric disorders.

Disclosures: **E.M. Parker:** None. **N.L. Kindja:** None. **C.E. Cheetham:** None. **R.A. Sweet:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.08/C5

Topic: B.07. Synaptic Plasticity

Support: The Project is supported by the European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.2- 16-2017-00012,
project title: Development of a product chain model for functional, healthy and safe foods from farm to fork based on a thematic research network.
B.R. is also supported by the János Bolyai Research Fellowship from the Hungarian Academy of Sciences and by the ÚNKP-18-4 New National Excellence Program of the Ministry of Human Capacities

Title: Effect of spermidine on the aging hippocampus: A quantitative electron microscopic study of synaptic and mitochondrial ultrastructure

Authors: *B. RACZ[1], S. SCHROEDER[2,3], S. HOFER[2,3], M. G. MARCELLO[1], P. T. SOTONYI[1], K. IWATA[4,5], S. J. SIGRIST[6], S. KIECHL[7], T. EISENBERG[2,3,8], F. MADEO[2,3]; [1]Dept. Anat. and Histology, Univ. of Vet. Med., Budapest, Hungary; [2]Inst. of Mol. Biosciences, Univ. of Graz, Graz, Austria; [3]BioTechMed-Graz, Graz, Austria; [4]Res. Ctr. for Child Mental Development,, Univ. of Fukui, Fukui, Japan; [5]Venetian Inst. of Mol. Medicine,, Padova, Italy; [6]Inst. of Biol, Freie Univ. Berlin, Berlin, Germany; [7]Dept. of Intrnl. Med. I, Gastroenterology, Endocrinol. and Metabolism, Med. Univ. of Innsbruck, Innsbruck, Austria; [8]Central Lab. Gracia, NAWI Graz, Univ. of Graz, Graz, Austria

Abstract: With increasing life expectancy age-associated cognitive decline is a prominent problem. Spermidine, a naturally occurring autophagy-inducing polyamine, is showing promising results of alleviating, and even reversing such age-associated cognitive decline. Dietary spermidine can pass the blood-brain barrier where it acts in part to promote spatial memory related cognition. We studied the potential neuroprotective effects of spermidine in aged mice. We targeted the CA1 region of the hippocampus with electron microscopy to quantify synaptic ultrastructure changes between young, old, and spermidine treated aged mice. We found improved morphological markers of synaptic plasticity in the CA1 stratum radiatum of spermidine treated aged mice compared to aged controls. As spermidine also promotes

mitophagy, we quantified potential changes in mitochondria: measured mitochondrial density in the neuropil, and quantified cristae width. We found that spermidine-treatment restored mitochondrial abundance, and prevented age-associated disorganization of cristae in the hippocampal CA1 region of aging mice. Our data suggest that nutritional spermidine may be therapeutically employed against age-associated cognitive decline.

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Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.09/C6

Topic: B.07. Synaptic Plasticity

Support: Davidson Research Initiative

Title: Neuroplasticity of the crossed temporodentate and septodentate pathways after entorhinal cortex injury in female rats

Authors: H. DOYLE, A. GHOSH, G. SODEN, K. BARLIS, ***J. J. RAMIREZ**;
Psychology and Neurosci., Davidson Col., Davidson, NC

Abstract: Alzheimer's disease (AD) is a debilitating neurodegenerative disorder characterized by memory loss and other behavioral problems. One of the principal early targets of AD is the entorhinal cortex (EC), a primary cortical input to the hippocampal formation. When the hippocampus is deafferented because of EC degeneration resulting from AD, several remaining afferents to the hippocampus undergo axonal sprouting. An established model to explore this concept in rats involves making a unilateral lesion of the EC, which elicits a sprouting response in fibers from the intact EC to the denervated contralateral dentate gyrus (DG) of the hippocampus, the so-called crossed temporodentate (CTD) pathway, as well as the septodentate pathway. Greater synaptic efficacy of the CTD has been found to occur as early as 6 days postlesion and increases in septodentate innervation have been observed as early as 5 days postlesion. To date, this model has been used almost exclusively in male rats, so it remains unclear whether the female rat brain evidences a similar kind of plasticity. The present study explored the nature of synaptic plasticity in the CTD and the septodentate pathway in female rats 12 days postlesion. Male and female Sprague-Dawley rats received either unilateral EC lesions or sham operations. For a paired-pulse assessment, a stimulating electrode was placed in the contralateral intact EC 12 days after a lesion or sham operation, and evoked field excitatory postsynaptic potentials (fEPSPs) were recorded in the DG ipsilateral to the lesioned EC. The

paired-pulse paradigm involved one pulse to the EC, known as the "conditioning pulse," followed by a second "test" pulse at a range of interpulse intervals (IPIs; 10 to 500 ms). The acetylcholinesterase (AChE) Naik stain was used to assess sprouting in the septodentate pathway in these cases. Additionally, estrous phases of female rats were recorded by lavages on both operation days. Electrophysiological analyses showed enhanced, lesion-induced, synaptic efficacy in the female CTD, similar to that seen in the male. Interestingly, histological analyses showed a significant main effect for lesions ventrally, but not dorsally for both sexes. Dorsally, females with lesions showed higher optical density values for AChE staining than male shams. Our findings suggest that EC lesions enhanced synaptic efficacy and evoked increases in AChE density in both sexes at 12 days postlesion. The septodentate sprouting response, however, appears to have been more vigorous dorsally in the female.

Disclosures: **J.J. Ramirez:** None. **H. Doyle:** None. **A. Ghosh:** None. **G. Soden:** None. **K. Barlis:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.10/C7

Topic: B.07. Synaptic Plasticity

Support: NIH R21 NS0902019
NIH RF1 MH114103
NIH T32 NS 086749
Kaufman Foundation

Title: Quantitative fluorescent synapse analysis reveals transient layer-specific reduction in PV inputs during sensory association training

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Abstract: Quantitative analysis of synaptic input is well-suited to evaluate changes in cortical circuitry during learning. Prior studies have suggested that parvalbumin (PV) inputs to neocortical pyramidal (Pyr) neurons are regulated by target cell activity and can be particularly labile during either sensory deprivation or with selective stimulation. Using an automated home-cage system for whisker-dependent sensory-reward association training (SAT) in freely-moving mice, we examined how PV inputs might be altered during sensory learning. We used neuroligin-based, postsynaptic labeling reagents (FAPpost) combined with PV-neurite labeling

for automated fluorescence detection of postsynaptic PV inputs onto L2 and L5 Pyr neurons. After 24-hr SAT, PV input density to both the soma and dendrites of L2 Pyr neurons was reduced by ~40%, while PV input density to L5 Pyr neurons was unchanged. Anatomical results were confirmed using analysis of PV channelrhodopsin-driven IPSCs in Ai32 x PV-Cre mice. PV input reduction was transient and renormalized after 5 days of continuous training. Use of other postsynaptic labeling reagents (gephyrin-FingRs) did not show reliable association with PV neurites. These results show that quantitative, input-specific synapse analysis can reveal experience- and context-dependent reorganization of synapses, validated by electrophysiological findings. Rapid changes in PV inputs to L2 Pyr neurons during sensory learning may facilitate subsequent plasticity across the cortical column.

Disclosures: D.A. Kuljis: None. **S.E. Myal:** None. **E. Park:** None. **K. Bregna:** None. **W. Wegner:** None. **K.I. Willig:** None. **A.L. Barth:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.11/C8

Topic: B.07. Synaptic Plasticity

Support: NIH Grant K99DA037279

Title: Behavioral sensitivity to acute and chronic opioid exposure in neuroligin-3 knockout mice

Authors: *D. D. BRANDNER, P. G. MERMELSTEIN, P. E. ROTHWELL;
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Abstract: The neuroligins are a family of postsynaptic cell-adhesion molecules that interact with presynaptic partners, the neurexins, to orchestrate the formation and functional properties of central synapses. Polymorphisms in these synaptic adhesion molecules have been shown to increase impulsivity and addiction-associated behaviors in human populations. The nucleus accumbens is a key neural substrate of addiction, and chronic exposure to drugs of abuse produces alterations in synapse structure and function in this brain region. Interestingly, nucleus accumbens synaptic function is also modified by depletion of specific neuroligin family members. Neuroligin-3 (NL3) is of interest because it participates in the organization of both excitatory and inhibitory synapses. Previous work has shown that NL3-knockout (KO) reduces the frequency of inhibitory synaptic currents in D1-medium spiny neurons (MSNs) of the nucleus accumbens, leading to an overall change in nucleus accumbens output that resembles the effects of chronic drug exposure. However, the mechanism by which NL3 modulates synaptic function remains incompletely understood, and a possible role for NL3 in mediating addiction-related behaviors in the nucleus accumbens has not been explored. To characterize the effects of

NL3 deletion on accumbens circuitry, we began by examining the anti-nociceptive and psychomotor activating effects of morphine in constitutive NL3 KO animals. Following acute morphine administration, these animals exhibited an increase in locomotor activity similar in magnitude to wild-type littermates. However, psychomotor sensitization was significantly reduced in NL3 KO animals following one week of morphine administered at 20 mg/kg daily. We are currently investigating the possibility that this altered behavioral response to morphine is related to structural alterations at excitatory and/or inhibitory synapses. We are also testing the behavioral effects of NL3 KO on the rewarding properties of morphine. In conjunction with these experiments, we are evaluating whether NL3 loss in specific subtypes of nucleus accumbens MSNs recapitulate aspects of the altered morphine response in global knockouts. Understanding the effects of NL3 depletion on synaptic architecture and nucleus accumbens circuitry may provide insight into pathologic alterations that influence human vulnerability to substance use disorders.

Disclosures: D.D. Brandner: None. **P.G. Mermelstein:** None. **P.E. Rothwell:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.12/C9

Topic: A.04. Transplantation and Regeneration

Support: NIH U01 MH105948
NIH R01 MH106507
NIH DP2 MH100011
Simons Foundation 399853
NIH R01 MH081880
NIMH R37 MH049428

Title: Interneuron transplantation creates new network states and rescues social behavior deficits in a mouse model of autism with excessive synaptic inhibition

Authors: *D. G. SOUTHWELL[1], H. SEIFIKAR[2], R. MALIK[3], K. LAVI[5], D. VOGT[6], J. L. RUBENSTEIN[4], V. S. SOHAL[7];
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Abstract: Manipulations that enhance GABAergic inhibition have been associated with improved behavioral phenotypes in autism models, suggesting that autism may be treated by correcting underlying deficits of inhibition. Interneuron transplantation is a method for increasing recipient synaptic inhibition, and it has been considered a prospective therapy for conditions marked by deficient inhibition, including neuropsychiatric disorders. It is unknown, however, whether interneuron transplantation may be therapeutic only for conditions marked by reduced inhibition, and, it is also unclear whether transplantation improves behavioral phenotypes solely by normalizing underlying circuit defects. To address these questions, we studied the effects of interneuron transplantation in mice lacking the autism-associated gene, *Pten*, in GABAergic interneurons. *Pten* mutant mice exhibit social behavior deficits, elevated synaptic inhibition in prefrontal cortex, abnormal baseline and social interaction-evoked electrocencephalogram (EEG) signals, and an altered composition of cortical interneuron subtypes. Transplantation of wild type embryonic interneurons from the medial ganglionic eminence into the prefrontal cortex of neonatal *Pten* mutants rescued social behavior despite exacerbating excessive levels of synaptic inhibition. Furthermore, transplantation did not normalize recipient EEG signals measured during baseline states, but it altered EEG responses observed during periods of social interaction. Interneuron transplantation can thus correct behavioral deficits even when those deficits are associated with elevated synaptic inhibition. Moreover, transplantation does not exert therapeutic effects solely by restoring wild type circuit states. Our findings indicate that interneuron transplantation could offer a novel cell-based approach to autism treatment, and they challenge assumptions that effective therapies must reverse underlying circuit defects.

Disclosures: D.G. Southwell: None. **H. Seifikar:** None. **R. Malik:** None. **K. Lavi:** None. **D. Vogt:** None. **J.L. Rubenstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurona Therapeutics. **V.S. Sohal:** 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.; Neurona Therapeutics.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.13/C10

Topic: B.07. Synaptic Plasticity

Support: CONACYT Grant 256882

Title: Chronic restraint stress induces anxiety & alteration of neuronal morphology in the centromedial amygdala in rats

Authors: *S. MORENO MARTÍNEZ[1,2], H. TENDILLA BELTRÁN[3], V. SANDOVAL[5], G. FLORES[4], J. TERRÓN SIERRA[2];
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Abstract: The centromedial amygdala (CeM) has efferent projections to the hypothalamus and is involved in the control of the stress response by modulating the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Hyperactivity of the amygdala has been associated with the endocrine and behavioral alterations of stress-related disorders, such as major depression. However, information regarding the impact of chronic stress on the CeM is scarce. The aim of the present study was to analyze the effect of 14-day chronic restraint stress (CRS; 20 min/day), as compared to home cage control (CTRL) conditions, on anxiety behavior and morphology of pyramidal neurons of the CeM in rats. Animals submitted to CRS displayed anxious behavior in both the elevated plus maze (EPM) and open field behavioral tests as compared to CTRL rats. In addition, CRS exposure decreased neuronal density as well as the number of dendritic spines in the CeM. Regarding the morphology of dendritic spines, CRS exposure reduced the percentage of mushroom-type spines along with an increase in the thim-type spines. Results suggest that CRS-induced anxiety-like behavior might be accounted for by neuronal morphological changes in the CeM.
Supported by the CONACYT (grant No. 256882)

Disclosures: S. Moreno Martínez: None. **H. Tendilla Beltrán:** None. **V. Sandoval:** None. **G. Flores:** None. **J. Terrón Sierra:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.14/C11

Topic: B.07. Synaptic Plasticity

Support: NIH-NIGMS: 2P20GM103642 (COBRE)
2R25GM061838-18 (RISE)
5R25GM061151-17 (RISE)

Title: The transcription factor gooseberry, a pax3/7 homolog, interacts with wingless (Wnt) to maintain neuronal function

Authors: *M. PEREZ CARAMBOT, C. DOMINICCI-COTTO, B. MARIE;
Inst. of Neurobio., San Juan, PR

Abstract: Transcription factors are important for nervous system development. They determine where neuroblasts develop (spatial selectors), their differentiation through time (temporal selectors), their cell fate (tissue/cell type selectors) and their final functional identity (terminal selectors). It has been shown that the activity of several terminal selectors persists in the mature brain to maintain neuronal sub-identity. This raises the question: can transcription factors also have a role to maintain basic general neuronal properties such as synaptic growth, plasticity and stability. We focus our study on Gooseberry (Gsb), a paired homeodomain transcription factor homologous to the vertebrate pax3/7. During early developmental stages, Gsb acts as a pair rule developmental regulator and controls the differentiation of a subset of neuroblasts. Interestingly, its role has been linked to its ability to antagonize the Wingless (Wg/Wnt) signaling. Here, we hypothesize that Gsb is required in mature motoneurons (MNs) to maintain general neuronal properties by antagonizing Wg. To assess the role of *gsb* in the mature nervous system we manipulated its expression at different stages of MN development and asked whether the growth, stability and plasticity of the neuromuscular junction was affected. Perturbing Gsb expression at early (post-mitotic; embryo) and late (after initial synaptic growth; larval stages 2 and 3) stages of synapse development affected synaptic growth, stability and plasticity suggesting that Gsb is not only an early fate determinant but is also required late to control MNs synaptic properties. In addition, we analyzed these synaptic properties after manipulating Gsb and Wg expression simultaneously. We found that Gsb antagonizes Wg to control synaptic growth and plasticity but controls synaptic stability independently of Wg. Gsb overexpression reduces synaptic growth and impairs synaptic plasticity, while Wg overexpression leads to overgrown and overplastic synapses. When Gsb and Wg are overexpressed simultaneously, the phenotypes are identical to the Gsb over expressors suggesting that Gsb renders the synapse resistant to Wg. In contrast, when we overexpress Gsb and activate the Wg pathway by expressing a dominant negative form of the kinase shaggy (Sgg), we found that both growth and plasticity phenotypes are restored to control levels. This finding strongly suggests that Gsb inhibits the Wg signaling pathway upstream of Sgg and downstream of Wg. We conclude that Gsb and Wg, two molecules essential to nervous system development, interact to control mature neuronal function.

Disclosures: M. Perez Carambot: None. **C. Dominicci-Cotto:** None. **B. Marie:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.15/C12

Topic: B.07. Synaptic Plasticity

Support: NIH R01 NIAID

Title: Microglia contribute to the loss of inhibitory synapses in chronic *Toxoplasma gondii* infection

Authors: *G. L. CARRILLO[1], T. GLAUSEN[3], J. TEAMER[2], Z. BOONE[2], I. BLADER[4], M. A. FOX[2];
[1]Fralin Biomed. Res. Inst., Roanoek, VA; [2]Fralin Biomed. Res. Inst., Roanoke, VA; [3]Univ. at Buffalo, Buffalo, NY; [4]Microbiology and Immunol., Univ. At Buffalo, Buffalo, NY

Abstract: Schizophrenia is a complex and heterogenous neurological disorder associated with debilitating cognitive impairment, acquisition of positive symptoms (such as hallucination and psychosis), and loss of behaviors that are normally present in healthy individuals (apathy and social withdrawal). Evidence from both human patients and rodent models suggest that schizophrenia-associated behaviors result from alterations in the assembly and function of inhibitory synapses, including inhibitory axo-somatic synapses. In addition to genetic causes (such as those examined with genetic mouse models) environmental factors can both increase the risk of schizophrenia and alter inhibitory circuit function in the brain. One such environmental factor is infection with *Toxoplasma gondii*, an intracellular protozoan parasite that infects over one-third of the human population worldwide. We previously discovered abnormalities in inhibitory synapse organization and function in chronically *Toxoplasma*-infected brains. Here, we sought to test whether chronic *Toxoplasma* infection specifically alters axo-somatic inhibitory synapses. We performed ultrastructural analysis of inhibitory axo-smatic synapses in the CA1 region of mouse hippocampus and in layer V of cerebral cortex using Serial Block Face Scanning Electron Microscopy (SBFSEM). The unique ultrastructural morphology of axo-somatic inhibitory synapses in these regions allowed us to unambiguously identify these synapses. In parasite-infected brains we discovered a significant reduction of inhibitory axo-somatic synapses in both CA1 and neocortex. Interestingly, we also observed a dramatic ensheathment of neuronal somas in these regions by microglia-like cells in *Toxoplasma*-infected brains. These findings were further corroborated with *in situ* hybridization for *Syt1* (a marker for neuronal somas) coupled with immunohistochemistry to visualize phagocytic microglia, along with a CX3CR1-GFP transgenic mouse model in which microglia are fluorescently labeled. Thus, we not only identified a significant reduction in axo-somatic synapses in parasite-infected brains, but our data suggests a role for microglia in inhibitory synapse loss.

Disclosures: G.L. Carrillo: None. **T. Glausen:** None. **J. Teamer:** None. **Z. Boone:** None. **I. Blader:** None. **M.A. Fox:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.16/C13

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NIM-HD MD007579
NIHGMS/INBRE P20 GM103475-14

Title: Combining optogenetics and FACS to examine NMDA subunit expression at specific synapses

Authors: *Y. CASTILLO-OCAMPO, A. HERNANDEZ-LOPEZ, M. COLON-ROMERO, P. LOPEZ, J. T. PORTER;
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Abstract: Post-traumatic stress disorder (PTSD) is proposed to occur due to miscommunication within the fear expression circuit which includes the amygdala, medial prefrontal cortex (mPFC) and hippocampus (HPC). Using optogenetics and brain slice recordings in adult male rats, our laboratory found a reduction of NMDA receptor-mediated currents at ventral hippocampal synapses onto infralimbic mPFC neurons (vHPC-to-IL synapses) after fear conditioning which had to be reversed to form an extinction memory. In this study, we combined optogenetic labeling of vHPC projections with fluorescence-activated cell sorting (FACS) to examine synaptic NMDA receptor subunits on vHPC-to-IL synapses. We infused AAV expressing channelrhodopsin and EYFP into the ventral hippocampus of male and female rats and exposed them to auditory fear conditioning and extinction two months later. At the end of the behavioral analysis, we sacrificed the rats, isolated synaptosomes from IL tissue punches, and labeled the obligatory NMDA receptor subunit, NR1, with fluorescently conjugated antibodies. Then, we analyzed NR1 expression on the vHPC synaptic terminals expressing EYFP with FACS. Rats exposed to fear conditioning froze more than rats exposed to extinction or unpaired controls, but NR1 expression on vHPC-to-IL synapses was similar in all groups. However, we did observe less NR1 expression in females than males in all behavioral groups. Overall our data suggests that the reduction of NMDA current after fear conditioning was not secondary to fewer NMDA receptors at the synapses and could, instead, be due to a change in NMDA subunit composition.

Disclosures: Y. Castillo-Ocampo: None. **A. Hernandez-Lopez:** A. Employment/Salary (full or part-time):; PHSU. **M. Colon-Romero:** A. Employment/Salary (full or part-time):; PHSU. **P. Lopez:** A. Employment/Salary (full or part-time):; PHSU. **J.T. Porter:** A. Employment/Salary (full or part-time):; PHSU. 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.; PHSU.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.17/C14

Topic: B.07. Synaptic Plasticity

Support: NSF-CREST HRD-1137725
NIH RISE 5R25GM061151-17

Title: Autophagy, highwire and map kinases regulate the temperature dependence of synaptic growth at the *drosophila* neuromuscular junction

Authors: *K. M. DE LEON GONZALEZ, B. MARIE;
Inst. of Neurobio., San Juan, Puerto Rico

Abstract: Climate change and the rising global temperature can have devastating effects on the population of arthropods and insects. The decline in the population of insects can have broad negative outcomes for several ecosystems that humans rely on. It is crucial that we understand the effect of temperature on the nervous system. Using the *Drosophila* Neuromuscular Junction (NMJ), we asked how rearing temperatures (15°C, 20°C, 25°C, 29°C) can affect synaptic growth. We observed an increase in the number of synaptic boutons in animals reared at higher temperatures. Indeed, animals reared at 29°C had a 100% increase in synaptic growth when compared to animals reared at 15°C. Interestingly, we found that the number of boutons from the 1s motor neurons increased with temperature while the boutons from the 1b motor neurons remained constant. This result indicates that motor neurons might be differentially sensitive to the changes in temperature.
Next, we identified highwire (Hiw), a E3 ubiquitin ligase, as a key regulator of temperature-dependent synaptic growth. Hiw is a negative regulator of synaptic growth and a loss of function mutation in the Hiw gene induced a temperature-independent overgrowth phenotype. We then asked whether autophagy, a known negative regulator of Hiw, was involved in this regulation. Autophagy loss of function mutants showed a temperature-independent undergrowth suggesting that autophagy may control temperature-dependent synaptic growth. In addition, using lysotracker, we showed that the levels of autophagy within the larval CNS increased at higher rearing temperatures. We hypothesize that, at 29°C, there is little suppression of synaptic growth due to the high activity of autophagy and the consequent low activity of Hiw. In contrast, at 15°C, reduced autophagic activity provokes increased Hiw activity and suppresses synaptic growth. Finally, we identified the Map Kinases Wallenda (Wnd) and P38b, known targets of Hiw, as key regulators of the synaptic growth of animals reared at 29^{o}C. Indeed, at 29^{o}C, animals presenting a loss of function mutation in Wnd had reduced synaptic growth when compared to control animals. We also revealed a genetic interaction between Wnd and P38b regulating

synaptic growth of animals reared at 29ºC. In this study we identified a pathway in which Autophagy and Highwire regulate temperature-dependent synaptic growth. In addition, we identified a novel role for Wallenda and P38b in regulating the synaptic growth of animals reared at higher temperature.

Disclosures: K.M. De Leon Gonzalez: None. **B. Marie:** None.

Poster

039. Structural Plasticity and Circuit Remodeling I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 039.18/C15

Topic: B.07. Synaptic Plasticity

Support: Special Coordination Funds for Promoting Science and Technology
The Brain Mapping by Integrated Neurotechnologies for Disease Studies

Title: CRMP2 binding compound accelerates recovery from central nervous system

Authors: *S. JITSUKI[1], Y. KAWAKAMI[1], M. SATO[2], A. JITSUKI-TAKAHASHI[1], H. TADA[3], H. MASUYAMA[4], T. OKUDA[4], T. TAKAHASHI[1];
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Abstract: Damage to the central nervous system (CNS) causes severe neurological conditions, such as sustained sensory, motor, cognitive dysfunction and compromise work capacity and self-care. No pharmacological intervention that could foster recovery and complement current rehabilitation has yet been established as effective. Restoration of motor impairment after CNS damage is considered to be the result of compensative neural plasticity in spared neural circuit, and the Experience-dependent synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid) receptor (AMPAR) delivery underlies behaviors that require neural plasticity such as learning. We found that a small compound, edonerpic-maleate (also known as T-817MA), facilitated experience-driven synaptic glutamate AMPA receptor delivery and resulted in the acceleration of motor function recovery after cortical or spinal cord cryoinjury. Edonerpic bound to collapsin-response-mediator-protein 2 (CRMP2), a downstream molecule of semaphorin, and is thought to be related to synaptic plasticity and learning. Furthermore, we detected CRMP2-dependent activation of ADF/cofilin by edonerpic maleate in the plasticity-inducing condition. Indicating edonerpic could facilitate synaptic AMPAR delivery through the regulation of actin dynamics. Thus, edonerpic-maleate, a neural plasticity enhancer, could be a clinically potent small compound to accelerate rehabilitation after damage of CNS.

Disclosures: **S. Jitsuki:** None. **Y. Kawakami:** None. **M. Sato:** None. **A. Jitsuki-Takahashi:** None. **H. Tada:** None. **H. Masuyama:** None. **T. Okuda:** None. **T. Takahashi:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.01/C16

Topic: B.08. Intrinsic Membrane Properties

Support: KAKENHI 18K06514

Title: Evaluating the contribution of passive propagation on axonal afterdepolarization using hippocampal mossy fiber model

Authors: *H. KAMIYA;
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Abstract: As a prominent feature of the axonal action potential, afterdepolarization lasting for several tens of milliseconds often follows the fast repolarization phase of the action potential. Small but prolonged afterdepolarization may modulate the subsequent action potential and transmitter release from the axon terminals. Several mechanisms underlying the generation of axonal afterdepolarization have been suggested, including passive propagation of upstream action potentials, the opening of slow voltage-dependent Na^+ currents, accumulation of K^+ ions in the surrounding extracellular space, and activation of ionotropic autoreceptors. To evaluate the relative contribution of the passive propagation in axonal afterdepolarization, a series of numerical simulations were performed using a mossy fiber model where voltage-dependent Na^+ and K^+ conductance was removed from the distal part of the axon. Slow depolarization with a similar time course with afterdepolarization was left after blockade of Na^+ and K^+ conductance in the model, suggesting that a capacitive component due to passive propagation contributes substantially in axonal afterdepolarization. It has been shown that experimentally recorded afterdepolarization showed clear voltage-dependency upon changes in the initial membrane potential, obviously deviating from those expecting from voltage-independent nature of the capacitive component. The simulation showed that the activation of voltage-dependent K^+ current also contributes to the initial phase and shapes a characteristic waveform of axonal afterdepolarization with a voltage-independent component of passive propagation. These findings suggest that the capacitive component reflecting passive propagation of upstream action potential substantially contributes to the slow time course of axonal afterdepolarization, although voltage-dependent K^+ current involves in the initial phase and provides a characteristic voltage-dependency to axonal afterdepolarization.

Disclosures: **H. Kamiya:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.02/C17

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant R21 NS097899

Title: Human cortical organoids reveal delayed maturation of cellular electrophysiologic properties by chronic methadone exposure during early neural development

Authors: *W. WU[1], H. YAO[2], A. R. MUOTRI[3], G. G. HADDAD[4];
[2]Dept Pediat, [3]Pediatrics/Cellular Mol. Med., [4]Dept. of Pediatrics, [1]UCSD, La Jolla, CA

Abstract: In the past decade, the opioid crisis has become a national epidemic and the number of opioid-dependent pregnant women has been soaring. Studies have shown that offsprings of mothers with methadone abuse during pregnancy manifest, years later, abnormalities in cognitive and intellectual capacities, indicating that there are not only short term but also long-term deleterious sequelae from methadone exposure. Although opioids and their receptors have been well characterized, the epidemic of opioid abuse has unmasked how little we know about the effects of methadone exposure on fetal brain development, short and long term. Cortical organoid derived from human iPSCs represents a cutting-edge model for the study of early brain development. Since organoids can be maintained and developed for long periods, we leveraged this opportunity and investigated the longitudinal effect of methadone exposure on membrane excitability in neurons and glia during early neural development. Methadone at clinically relevant concentrations (1 μM or 10 μM) were added to the culture medium for organoids starting from 12-weeks of age. We performed parallel patch-clamp recordings of neurons from 16-, 20-, and 24-week-old cortical organoids for control and methadone-treated groups. During this developmental time, we observed a progressive increase of neural size, # of action potential (AP) firing, and single AP properties including the AP amplitude, the maximal AP rise/decay slopes, and the AHP amplitude in control groups, indicating a 'maturing' process of neuronal excitability. However, chronic exposure to 1 μM and 10 μM methadone produced a significant delay in neural size, # of AP firing, and single AP properties. For example, from 12-week to 20-week-old organoids, # of AP firing (evoked by 50 pA for 1000ms) was increased from 1.7±1.2 to 12.6±0.8 in control groups, but only increased to 7.2±1.3 and 2.5±0.3 for 1 μM and 10 μM methadone groups, respectively ($p<0.01$, as compared to control). We further used voltage-clamp techniques to determine the developmental changes of specific ion channels. From 12-week till 24-week, we observed progressive increases of voltage-gated Na^+ current and K^+ current in control groups, but a significant delayed increase in methadone treated groups. Overall, human cortical organoids revealed that long-term methadone exposure significantly delay the growth of

neuronal excitability due to the attenuated function of voltage-gated Na^+ and K^+ channels. The findings suggest that chronic methadone exposure could potentially lead to a longitudinal impairment of membrane properties and excitability during early neural development.

Disclosures: W. Wu: None. **H. Yao:** None. **A.R. Muotri:** None. **G.G. Haddad:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.03/C18

Topic: B.08. Intrinsic Membrane Properties

Support: CIHR grant 153143
NSERC grant 04336

Title: Biophysical signatures of sparse coding in hippocampal-like circuits

Authors: *A.-T. TRINH[1], S. E. CLARKE[2], E. HARVEY-GIRARD[1], L. MALER[1];
[1]Dept. of Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; [2]Dept. of Bioengineering,, Stanford Univ., Stanford, CA

Abstract: Several studies in teleosts, including those in gymnotiform fish, have shown that fish can also learn the spatial relations between distinct landmarks. Early lesion studies in the goldfish dorsal lateral pallium (DL) have shown that it is necessary for spatial learning. In the gymotiform fish, previous anatomical studies have suggested that DL is homologues to the mammalian hippocampus, in particular to the dentate gyrus (Elliott et al., 2017, J. Comp. Neurol.). However, DL has also drawn multiple comparisons to cortex since it receives extrinsic projections from teleost thalamus, the preglomerular nucleus (PG) and the DL microcircuitry suggests that its neurons form local (excitatory) recurrent networks (Yamamoto et al., 2007, Brain Behav. Evol.; Trinh et al., 2016, J. Comp. Neurol.).
More recently, a subset of electrosensory motion PG neurons in the gymnotiform fish was shown to encode the time interval between object encounters which led the authors to hypothesize that the fish can estimate the distance between objects (Wallach et al., 2018, eLife). Since the DL of gymnotiform fish receives both visual and electrosensory inputs from PG, we hypothesize that the transformation of electrosensory motion signals to a spatial map are processed in DL.
To test whether DL neurons may be able to perform this task, we performed *in-vitro* whole-cell patch recordings of neurons in DL of *Apteronotid* fish and of *Carassius auratus* (goldfish). We have found that DL neurons are very similar in both species: they have a hyperpolarized resting membrane potential that is likely due to the activity of GIRK channels, they exhibit a high spike threshold and they display a large small conducting Ca^{2+}-activated K^+ channel (SK)-mediated AHP; all these mechanisms contribute to the low current-evoked firing rate observed in these

neurons. Based on these biophysical characteristics, we propose that DL neurons may be sparse coders similarly to the granule cells of the mammalian dentate gyrus.
Furthermore, we also found that DL neurons exhibit prolonged spike threshold adaptation which, based on our model results, may be due to the slow cumulative inactivation of Na^+ channel. We propose that this spike threshold adaptation will allow the DL recurrent network to encode temporal sequences as suggested by the computational work of Istkov et al. (2011, Journal of Neuroscience). The DL network would then be able to decode the time information transmitted from the afferent thalamic input (Wallach et al., 2018, eLife) into an estimation of the animal's location as it swims from a landmark to food, thus providing the basic framework for the formation of a spatial map.

Disclosures: **A. Trinh:** None. **S.E. Clarke:** None. **E. Harvey-Girard:** None. **L. Maler:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.04/C19

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant 1S06GM067078-01A2
The Howard Hughes Medical Institute
UNCF-McBay Fellowship

Title: Different effects on primary afferent muscle spindle afferents vs golgi tendon organ afferents in short term hyperglycemic and insulin nanoparticle treated rats

Authors: *V. K. HAFTEL[1], C. SMITH[2];
[1]Biol., [2]Morehouse Col., Atlanta, GA

Abstract: Primary afferents innervating muscle of rats are susceptible to changes in function from injury and disease. In previous studies on the functional result of short-term uncontrolled hyperglycemia (3 weeks, 6 weeks) we showed muscle spindle afferents to have altered function, as measured by sciatic function index, reflex contraction, and number and maximum frequency of action potentials fired by individual axons recorded intra-axonally. It may be logical to assume that golgi tendon organ afferents (GTOs) display similar changes in function in the face of short-term hyperglycemia, but it is not known. In these studies, various parameters of individual neuron firing are examined in GTOs and compared across treatment groups (untreated, 3 week hyperglycemic (3wk), 6 week hyperglycemic (6wk), 3 week hyperglycemic + 1 week insulin nanoparticles (3wk/1wk); 3wk/2wk; and 2wk/2wk. GTO function was also compared to that of muscle spindle afferent firing parameters. These axons were randomly recorded from the same anesthetized animals in the same treatment groups and categorized based

on typical firing responses to stretch and muscle twitch response to nerve stimulation (1 Hz). GTOs do not significantly change function across groups when comparing number of action potentials, maximum instantaneous firing rate, threshold length of firing in 3wk and 6wk compared to untreated, although the values did increase in the 3wk group ($p > 0.05$, ANOVA post hoc Unequal N Honest Significant Difference). When exposing muscle to insulin-containing nanoparticles for various durations listed above, the values also did not change significantly across treatment groups for GTOs, indicating that the injections are neither improving nor harming function in these specific primary afferents. This is contrary to the findings for muscle spindle afferents, in which function was significantly increased at 3wk, decreased at 6wk and improved with nanoparticle injection (3wk/1wk). The difference in resulting function in the treatment groups for the two types of afferents may be due to the nature and location of the sensory ending and their distinct interaction with muscle tissue. Future studies will examine muscle tissue in these groups as well as the ultrastructure of the sensory ending.

Disclosures: V.K. Haftel: None. **C. Smith:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.05/C20

Topic: B.08. Intrinsic Membrane Properties

Support: PAPIIT IA206317

Title: Electrophysiological characterization of NPY and POMC neurons of the hypothalamic arcuate nucleus

Authors: D. CASTILLO-ROLON, S. ORTEGA-TINOCO, N. VILLALOBOS, G. ARENAS-LOPEZ, S. HERNANDEZ-LOPEZ, ***J. GARDUÑO**;
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Abstract: The regulation of food intake and energy expenditure are under the control of two main populations of neurons in the arcuate nucleus (ARC), which is localized in the base of the hypothalamus. The NPY neurons are orexigenic and their activation stimulates food intake. On the other hand, the activation of POMC neurons has an anorectic effect. Several works have tried to identify these neurons on the base of their electrophysiological and pharmacological properties. However, the classification of ARC neurons is still inconclusive. We used brain slices obtained from male Wistar rats (18-21 postnatal days) and performed whole-cell patch recordings to determine the electrophysiological differences among these types of cells. We changed glucose concentrations within a physiological range and recorded the electrical response to identify them. In addition, cells were identified by immunocytochemical techniques by using

anti-NPY and anti-MSH antibodies. The ARC neurons were classified based on their passive and active electrophysiological properties. Also, we performed calcium imaging experiments to record the response of dozens of cells simultaneously with single cell resolution. We observed that NPY and POMC neurons have different electrophysiological properties, such as input resistance and firing pattern, and can be distinguished based on these characteristics. The diversity of ARC neurons based on their response to different glucose concentrations was confirmed with calcium imaging experiments.

Disclosures: D. Castillo-Rolon: None. **S. Ortega-Tinoco:** None. **N. Villalobos:** None. **G. Arenas-Lopez:** None. **S. Hernandez-Lopez:** None. **J. Garduño:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.06/C21

Topic: B.08. Intrinsic Membrane Properties

Support: NIH-NEI R01DY027380

Title: Intrinsic excitability of neurons of the xenopus laevis optic tectum is regulated by changes in sodium currents during retinotectal circuit development

Authors: *A. C. THOMPSON, C. D. AIZENMAN;
Neurosci., Brown Univ., Providence, RI

Abstract: During the development of the *Xenopus laevis* retinotectal circuit the level of synaptic input received by tectal neurons changes dramatically as activity-dependent synaptic refinement and strengthening occurs, yet tectal neurons maintain the capacity to stably respond to visual stimulation. To maintain a stable relationship between synaptic input and spike output, the intrinsic excitability of tectal neurons decreases as the retinotectal circuit matures. One mechanism by which neurons can regulate intrinsic excitability is the modulation of voltage-dependent sodium currents; however, the mechanisms by which sodium currents are regulated to modulate intrinsic excitability of tectal neurons remains largely unknown.
To determine whether sodium currents are modualted as intrinsic excitability of tectal neurons changes, we measured sodium currents with whole-cell patch clamp electrophysiology across a time-course of retinotectal circuit development and in response to enhanced network activity. Here we describe how fast, persistent and resurgent sodium current conductance are regulated, revealing that sodium currents are enhanced congruent with increased intrinsic excitability. To examine how sodium currents are modulated as intrinsic excitability of tectal neurons changes, we quantified the expression of voltage-gated sodium channel subtypes across retinotectal circuit development and in response to enhanced network activity. We found that expression of voltage-

gated sodium channel subtypes $Na_v1.1$, $Na_v1.2$ and $Na_v1.6$, as well as the accessory $Na_v\beta4$ subunit, are differentially regulated across development and in response to enhanced network activity. Finally, we explore the contribution of voltage-gated sodium channel subtypes to fast, persistent and resurgent sodium currents and the intrinsic excitability of tectal neurons.

Disclosures: A.C. Thompson: None. **C.D. Aizenman:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.07/C22

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant R01062771
NIH Grant R25GM109439
NIH Grant T32MH020065

Title: Metabotropic receptor signalling facilitates intrinsic plasticity in cerebellar Purkinje cells

Authors: *G. WATKINS[1], C. HANSEL[2];
[1]Univ. of Chicago, Chicago, IL; [2]Neurobio., Univ. of Chicago Dept. of Neurobio., Chicago, IL

Abstract: Intrinsic plasticity - experience-dependent changes in the membrane excitability of individual neurons - has been identified as an important mechanism of learning and formation of memory engrams. In the cerebellum, Purkinje cells generate the sole output of the cerebellar cortex and regulate motor learning. Intrinsic plasticity (IP) in Purkinje cells is mediated in part through the downregulation of small conductance calcium-activated potassium (SK) channels, which are involved in regulation of the medium afterhyperpolarizing potential. In other brain areas, SK channel trafficking is regulated by the activity of muscarinic acetylcholine receptors (mAChRs). In the cerebellum, mAChR expression is limited to lobules IX and X, the vestibulocerebellum. Using whole-cell patch-clamp recordings from Purkinje cells in sagittal slices prepared from the vermis of young adult (P21-42) mice, we demonstrate that mAChRs also facilitate intrinsic plasticity in the cerebellum. Bath application of the general mAChR agonist oxo-m significantly increased the evoked firing rate of Purkinje cells in lobules IX/X in response to a physiologically relevant synaptic induction protocol at the parallel fiber-Purkinje cell synapse (syn+oxo-m, firing increased 178.8 ± 11%, $n = 9$, $p < 0.05$) as compared to IP expression induced by either the synaptic protocol or oxo-m application alone (syn IX/X, firing increased 141.5% ± 9%, $n = 11$, $p < 0.05$; oxo-m control, firing increased 116.8 ± 6.2%, $n = 9$, $p < 0.05$). While mAChR expression is restricted to the vestibulocerebellum, type 1 metabotropic glutamate receptors (mGluR1s) are expressed throughout the cerebellum and share the Gq-protein coupled signaling pathway with mAChRs. Bath application of the group I mGluR agonist

DHPG significantly increased the IP amplitude throughout the cerebellum (syn+DHPG firing increased 192.9 ± 22%, n = 5; $p < 0.05$), relative to the synaptic protocol alone (syn I-VIII, firing increased 135.6% ± 13%, n = 6, $p < 0.05$). These findings suggest that Gq-coupled receptors may be involved in modulating the activity of SK channels and enhancing membrane excitability in Purkinje cells.

Disclosures: G. Watkins: None. **C. Hansel:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.08/C23

Topic: B.08. Intrinsic Membrane Properties

Support: R35NS097212

Title: Dual separable feedback systems govern firing rate homeostasis

Authors: *Y. KULIK[1], R. T. JONES[3], A. MOUGHAMIAN[2], J. WHIPPEN[2], G. W. DAVIS[4]; [1]Biochem. and Biophysics, [2]UCSF, San Francisco, CA; [3]Univ. of California, San Francisco, San Francisco, CA; [4]Dept Biochem & Biophysics, Univ. California-SF, San Francisco, CA

Abstract: Firing rate homeostasis (FRH) stabilizes neural activity. A pervasive and intuitive theory argues that a single variable, calcium, is detected and stabilized through regulatory feedback. A prediction is that ion channel gene mutations with equivalent effects on neuronal excitability should invoke the same homeostatic response. In agreement, we demonstrate robust FRH following either elimination of Kv4/Shal protein or elimination of the Kv4/Shal conductance. However, the underlying homeostatic signaling mechanisms are distinct. Eliminating Shal protein invokes Krüppel-dependent rebalancing of ion channel gene expression including enhanced slo, Shab, and Shaker. By contrast, expression of these genes remains unchanged in animals harboring a CRISPR-engineered, Shal pore-blocking mutation where compensation is achieved by enhanced IKDR. These different homeostatic processes have distinct effects on homeostatic synaptic plasticity and animal behavior. We propose that FRH includes mechanisms of proteostatic feedback that act in parallel with activity-driven feedback, with implications for the pathophysiology of human channelopathies.

Disclosures: Y. Kulik: None. **R.T. Jones:** None. **A. Moughamian:** None. **J. Whippen:** None. **G.W. Davis:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.09/C24

Topic: B.08. Intrinsic Membrane Properties

Support: JST ERATO (JPMJER1801)
JSPS Grants-in-Aid for Scientific Research (18H05525)
the Human Frontier Science Program (RGP0019/2016)

Title: Excitability hysteresis of retrosplenial layer 2/3 pyramidal cells after subicular synaptic inputs

Authors: *M. GAO[1], A. NOGUCHI[1], Y. IKEGAYA[2];
[1]The Univ. of Tokyo, Tokyo, Japan; [2]Grad Sch. Pharma Sci, Univ. Tokyo, Tokyo, Japan

Abstract: The granular retrosplenial cortex (RSC) have structural connections with various brain regions, such as the hippocampal formation, the subiculum, the parietal cortex, and the limbic thalamus (Seralynne D Vann et al., Nat Rev Neurosci, 2009). Receiving inputs from these different areas, RSC has been implicated to process and integrate information so as to mediate diverse cognitive functions. In the present study, we investigated the potential circuit of projections to RSC layer 2/3 (L2/3) late-spiking neurons from the dorsal subiculum and local cortical areas (*i.e.*, RSC L5). We found that preceding brief optogenetic activation of subiculum-to-RSC L2/3 excitatory projections shortened the first spike latency of RSC L2/3 late-spiking neurons induced by rectangular current injections, while activation of local cortical excitatory projections delayed the first spike latency. Instead of the prior activation of subicular inputs, a brief current injection into the soma of RSC L2/3 late-spiking neurons also led to a shortening of the first spike latency, which was a unique phenomenon that does not occur in RSC L5 neurons, anterior cingulate cortex L2/3 neurons, or hippocampal CA1 neurons. In addition, the firing probability and the EPSP amplitudes in RSC L2/3 late-spiking neurons caused by local field stimulation were also enhanced by prior optogenetic activation of subicular inputs. Consequently, subicular and local cortical projections to RSC L2/3 late-spiking neurons oppositely modulate the spike timing, which possibly correlates to the encoding different contexts. We will further conduct investigation under in vivo conditions.

Disclosures: **M. Gao:** None. **A. Noguchi:** None. **Y. Ikegaya:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.10/C25

Topic: B.08. Intrinsic Membrane Properties

Support: NINDS grant P50 NS047085
NINDS grant R37 NS041280
NINDS grant F31 NS100357

Title: Dopaminergic transmission rapidly and persistently enhances excitability of D1 receptor-expressing striatal projection neurons

Authors: *A. LAHIRI, M. BEVAN;
Physiol., Northwestern Univ., Chicago, IL

Abstract: Substantia nigra dopamine neuron activity has recently been linked to the initiation and invigoration of movement on the sub- to multi-second timescale. Dopaminergic modulation of striatal projection neuron (SPN) activity is thought to underlie this linkage, although the impact of native transmission on SPN excitability has not been directly demonstrated. Using perforated patch-clamp recording, we found that optogenetic stimulation of nigrostriatal dopaminergic axons rapidly and persistently elevated the intrinsic excitability of dopamine D1 receptor-expressing SPNs (D1-SPNs). The evoked firing of D1-SPNs increased within hundreds of milliseconds of stimulation and remained elevated for several minutes thereafter. Consistent with the negative modulation of depolarization- and Ca^{2+}-activated K^{+} currents, dopaminergic transmission accelerated subthreshold depolarization in response to somatic current injection, reduced the latency to fire, and diminished action potential afterhyperpolarization. These data demonstrate that dopaminergic transmission potently increases D1-SPN excitability with a time course that could support both sub-second and sustained behavioral control.

Disclosures: A. Lahiri: None. **M. Bevan:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.11/C26

Topic: B.08. Intrinsic Membrane Properties

Support: NIH R01 MH109471

Title: Comparison of female rat nucleus accumbens core neuron electrophysiological properties between early and late proestrus

Authors: *S. PROANO, A. KRENTZEL, J. MEITZEN;
North Carolina State Univ., Raleigh, NC

Abstract: The brain operates under a dynamic neuroendocrine environment. Naturally occurring hormone cycles in adult female humans and rodents induce sex-specific differences in neuron function across the brain, including many normal behaviors and disorders. This includes the phenotype and incidence of reward- and motivated-related behaviors, including relevant disorders such as addiction, anxiety, and depression. This implies that the instrumental neural substrate, which includes the nucleus accumbens core (AcbC), is likewise susceptible to the influence of ovarian hormones. Previous findings indicate that the electrophysiological properties of the AcbC's output neurons, medium spiny neurons (MSNs), differ robustly by estrous cycle stage in adult female rats and induce sex differences when compared to MSNs recorded in male rats. However, these data do not discriminate between early proestrus when estradiol levels peak and late proestrus when progesterone levels peak. Here we test whether the intrinsic electrophysiological properties of MSNs differ between early and late proestrus using the whole-cell patch clamp technique in gonad intact adult female rats. Analysis of intrinsic electrophysiological properties shows that action potential half-width and sag index differ between early and late proestrus. These findings indicate that electrophysiological properties important for MSN output differ between early and late proestrus, with resulting implications for nucleus accumbens mediated behaviors and disorders.

Disclosures: S. Proano: None. **A. Krentzel:** None. **J. Meitzen:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.12/C27

Topic: B.08. Intrinsic Membrane Properties

Support: Mercator Stiftung
German Research Foundation (DFG) project YO177/4-1

Title: Role of noradrenaline and serotonin in intrinsic persistent firing in CA1 pyramidal neurons

Authors: *A. REBOREDA[1,2], M. J. VALERO-ARACAMA[3,4], A. ARBOIT[2], M. SAUVAGE[1], M. YOSHIDA[1,2];
[1]Functional Architecture of Memory, Leibniz Inst. For Neurobio., Magdeburg, Germany;

[2]Cognitive Neurophysiol., German Ctr. For Neurodegenerative Diseases-DZNE, Magdeburg, Germany; [3]Ruhr Univ. Bochum, Bochum, Germany; [4]Dept. of Physiol. and Pathophysiology, Friedrich-Alexander-University of Erlangen-Nürnberg, Erlangen, Germany

Abstract: The correct performance of working memory tasks requires a fine-tuning of different neuromodulatory systems. It is accepted in general that working memory requires high cholinergic activation while other neuromodulators such as 5-HT and NA have mixed effects depending on the activation levels or the receptor subtypes involved. However, it remains unclear why these neuromodulators exert different effects.
In the present study, we focus on the effects of neuromodulators on the ability of individual neurons to support persistent firing, which is one of the potential cellular correlates of working memory. Using in vitro patch clamp recording in hippocampal CA1 pyramidal neurons. We first demonstrate that the cholinergic receptor subtypes, M1 and M2, support the generation of persistent firing in the presence of a cholinergic agonist, pointing out that both Gq and Gi coupled receptors are required to trigger PF.
On the other hand, application of 5-HT or NA by themselves did not support generation of persistent firing, while co-application of either of them with a cholinergic agonist suppressed cholinergically triggered persistent firing. Receptor subtype studies pointed out that 5-HT6 serotoninergic and β1 adrenergic receptors are responsible for the suppressive effect, while 5-HT7 and β2 did not affect the persistent firing significantly. These results suggest that an activation of Gs coupled receptors, which is detrimental for working memory in vivo, suppresses PF in CA1 pyramidal neurons.
We further explored the involvement of cAMP pathway using the adenylate cyclase activator and the PKA inhibitor. Our results suggest that the adenylate cyclase activator suppresses persistent firing while the PKA inhibition rescues persistent firing under the 5-HT activation, indicating the involvement of cAMP pathway downstream of the Gs receptor activation.
In summary, intrinsic persistent firing in CA1 pyramidal neurons requires Gq activation and reduced levels of cAMP. Activation of Gs coupled receptors, on the other hand, inhibits the cholinergic persistent firing through cAMP mediated activation of PKA. We will further discuss the similarity between the modulation of persistent firing and working memory by the neuromodulatory systems.

Disclosures: A. Reboreda: None. **M.J. Valero-Aracama:** None. **A. Arboit:** None. **M. Sauvage:** None. **M. Yoshida:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.13/C28

Topic: B.08. Intrinsic Membrane Properties

Support: UW-Milwaukee Research Growth Initiative

Title: Developmental emergence of sex differences in the intrinsic membrane properties of retrosplenial cortical neurons

Authors: *H. YOUSUF[1], J. R. MOYER, Jr.[2];
[1]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Psychology, Univ. of Wisconsin Milwaukee Dept. of Psychology, Milwaukee, WI

Abstract: The granular retrosplenial cortex (gRSC) forms reciprocal connections with the hippocampus to support contextual fear learning. Recent evidence has demonstrated clear sex differences in contextual fear learning where adult female rodents exhibit greater fear learning compared to adult males (Keiser et al., 2017; Asok et al., 2019). Moreover, sex differences in context fear conditioning emerge during preadolescence and follow a nonlinear developmental trajectory into adulthood (Colon et al., 2018). Despite its role in associative learning, little is known about the electrophysiological properties of gRSC neurons, and nothing is known about how they vary as a function of sex and age. Using visually-guided, patch-clamp recordings in brain slices, we characterized a distinctive population of regular spiking (RS) neurons in L5 of gRSC from naïve preadolescent (i.e., postnatal days 14-29) male and female rats. In response to increasing current injections, RS neurons in gRSC from male preadolescent rats fired significantly more action potentials compared to those from female preadolescent rats ($p < 0.05$). Significant differences in the electrophysiological properties emerged during adolescence (i.e., postnatal days 36-39). For example, unlike preadolescent neurons, the majority of recordings in L5 gRSC neurons from adolescent male and female rats had a prominent afterdepolarization (ADP) following a single action potential, and are characterized as regular-spiking ADP (RS_{ADP}) neurons. As observed in hippocampal neurons (Brown and Randall, 2009), the emergence of the ADP property enhances excitability in both adolescent male and female gRSC neurons. Compared to preadolescence, sex differences in neuronal excitability during adolescence were reversed such that adolescent female RS_{ADP} neurons exhibited significantly greater intrinsic excitability compared to adolescent male RS_{ADP} neurons ($p < 0.05$) in the gRSC. The ADP property in L5 gRSC remained prevalent in adults (~3 months) from both sexes. Interestingly, sex differences in the excitability of RS_{ADP} neurons in gRSC of adults were most similar to those observed during preadolescence. RS_{ADP} neurons in L5 of gRSC from adult male rats exhibited an enhanced intrinsic excitability compared to those from adult female rats ($p < 0.05$). Our findings suggest that the gRSC is a sexually dimorphic region and may undergo nonlinear changes across development. Understanding developmental patterns in fear-related brain structures may help practitioners optimize treatments for certain juvenile psychiatric disorders.

Disclosures: H. Yousuf: None. **J.R. Moyer:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.14/C29

Topic: B.08. Intrinsic Membrane Properties

Support: University of Idaho Seed Grant

Title: Spatiotemporal dopaminergic modulation of Schaffer collateral-CA1 plasticity: A computational modeling approach

Authors: *J. SCHMALZ, G. KUMAR;
Chem. and Materials Engin., Univ. of Idaho, Moscow, ID

Abstract: The complex, synergistic actions of stress-induced hormones such as corticosterone and neuromodulators such as dopamine impacts the dynamic activity of specific brain regions such as the hippocampus. Gaps in our understanding of this impact have hindered the optimal efficacy of existing therapies for stress-mediated disorders, such as post-traumatic stress disorder and major depressive disorders. Although the specific roles of stress-relevant hormones and neuromodulators in modulating synaptic plasticity and memory have been relatively well-investigated, how they act together to impact synaptic integrity, plasticity and memory is poorly understood. We developed an experimentally constrained computational modeling framework by integrating molecular-level dynamics with the electrophysiology of CA1 pyramidal (CA1Py) neurons to investigate the spatiotemporal effects of dopamine (DA) on the hippocampal Schaffer Collateral (SC)-CA1 long-term potentiation (LTP) / depression (LTD) and CA1Py neurons excitability. In the hippocampus, the release of DA is critical for memory consolidation and spatial memory. One of the ways memories may be regulated is through DA modulation of SC-CA1 LTP/LTD and CA1Py neuron excitability. Experimentally, it has been shown that activation of the D1/D5 receptor enhances SC-CA1 LTP, which is measured through an increase in the slope of the evoked excitatory postsynaptic potentials (EPSPs). This enhancement of potentiation is observed after LTP induction by high frequency stimulation (HFS) and LTD by low frequency stimulation (LFS) of the SC axon. In addition to modulating LTP/LTD on a time-scale of several minutes to hours, D1/D5 receptor activation controls the intrinsic excitability of a CA1Py neuron by modulating several ionic conductance at the time-scale of several minutes to an hour. The permeabilities of these ion channels are modulated by their phosphorylation level that is controlled by a cAMP dependent phosphorylation cascade gated by G-coupled metabotropic dopamine receptors. To capture the effect of DA on ligand- and voltage-gated ion channels, we developed a reduced DA dynamic modulation model that captures the enhancement of potentiation and changes in neuron excitability as a function of D1/D5 receptor activation. We incorporated this reduced model into our experimentally validated Hodgkin-Huxley model of a

single CA1Py neuron and investigated the effect of the complex temporal interaction between D1/D5 activation and HFS/LFS of the SC axon on LTP/LTD and CA1Py dynamics. Our modeling results show the temporal relationship between D1/D5 activation dynamics and HFS/LFS in altering CA1Py dynamics.

Disclosures: J. Schmalz: None. **G. Kumar:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.15/C30

Topic: B.08. Intrinsic Membrane Properties

Title: Assembly of excitable membrane domains at the neuro-cardiac sympathetic synapses

Authors: *O. G. SHCHERBAKOVA[1], T. HUND[2], P. MOHLER[3];
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Abstract: Sympathetic synapses between sympathetic neurons (SGN) and cardiac myocytes are unique cell-cell contacts designed for the efficient regulation of heart rate and contractility by the autonomic nervous system. In order to investigate functional connections of the neuro-cardiac junctions, we employed model system of co-cultures of neonatal cardiac myocytes and sympathetic neurons. We provided the first evidence for molecular specialization in the postsynaptic membranes of innervated cardiac myocytes in co-culture with sympathetic ganglion neurons(Shcherbakova O. et al., 2007), that was explored further by other authors (Prando V. al., 2018). In the present study, we report that ankyrin G is enriched at the postsynaptic sites in co-culture of cardiac myocytes and SGN. Ankyrin G is a scaffold protein known to interact with KCNQ and Na^+ voltage-gated channels and retain them at the excitable domains of neuronal membranes, such as axonal initial segments and nodes of Ranvier (Pan Z. et al., 2006). Consistent with this, we have found that KCNQ1 channels are present in increased density at the specialized zones of contacts of cardiac myocytes and SGN. We also have observed an increased density of Na^+ channels. Cardiac $Na_v1.5$ volgate-gated channels are responsible to upstroke of cardiac action potential, while KCNQ1 channels are involved in repolarization of cardiac myoyctes. Mutations in cardiac $Na_v1.5$ channel, that affect binding to the scaffold protein ankyrin G, cause Brugada syndrome, which is a severe form of arrhythmia leading to sudden death (Mohler P. et al., 2004). Variations in channels density and surface expression are known to contribute to the modulations of particular currents (Lai and Jan 2006). Therefore, an increased density of Na and KCNQ1 channels along with ankyrin G at the neuro-cardiac sympathetic synapses indicates that the excitable domains assembled at the neuro-cardiac

sympathetic synapses that may have an important role in modulation of cardiac action potential by neuronal input.

Disclosures: O.G. Shcherbakova: None. **T. Hund:** None. **P. Mohler:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.16/C31

Topic: B.08. Intrinsic Membrane Properties

Support: Research was supported by NIGMS (R01 GM097433) and U.S. Department of Veterans Affairs (BX002547).

Title: Sodium channel gating sensitivity to external calcium is regulated by the calcium-sensing receptor in neocortical neurons

Authors: *B. J. KNIGHT, S. M. SMITH;
PCCM, VAPORHCS/OHSU, Portland, OR

Abstract: Decreasing extracellular [Ca^{2+}] ([Ca^{2+}]$_o$) increases excitability in neurons. This universal effect has been attributed to a loss of Ca^{2+}-mediated screening of surface charge impacting voltage-dependent channel gating. In contrast, [Ca^{2+}]$_o$-dependent changes in excitability in hippocampal neurons were entirely disrupted by deletion of a specific pathway consisting of a non-selective cation channel (NALCN), and intracellular proteins UNC-79 and UNC-80. It was hypothesized that changes in [Ca^{2+}]$_o$ were detected by the Calcium-sensing receptor (CaSR), upstream of UNC-79, UNC-80, which activated NALCN triggering action potentials (AP). Using patch-clamp methods we tested if CaSR mediated [Ca^{2+}]$_o$-dependent excitability in neurons. We examined intrinsic excitability in pharmacologically isolated (glutamat- and GABAergic transmission blocked), cultured mouse neocortical neurons. AP firing rates increased significantly in cre-positive WT (creWT) neurons following the switch from physiological Tyrode ($T_{1.1}$; containing 1.1 mM [Ca^{2+}] and 1.1 mM [Mg^{2+}]) to reduced Ca^{2+} and Mg^{2+} Tyrode ($T_{0.2}$; containing 0.2 mM [Ca^{2+}] and [Mg^{2+}]), but not in conditional CaSR null mutant (creCasr$^{-/-}$); consistent with CaSR regulation of NALCN. However, creWT and creCasr$^{-/-}$ neurons had different resting membrane potentials. After correction for this, both genotypes similarly increased in firing after the switch from $T_{1.1}$ to $T_{0.2}$. Furthermore, creWT and creCasr$^{-/-}$ neurons both depolarized following the $T_{1.1}$ to $T_{0.2}$ switch indicating CaSR-NALCN mediated depolarization was unlikely to explain [Ca^{2+}]$_o$-dependent excitability. Characterization of the currents in voltage-clamped creWT and creCasr$^{-/-}$ neurons showed many differences. Most importantly, the voltage-gated sodium channel (VGSC) activation and steady-state inactivation curves were left-shifted for the creCasr$^{-/-}$ neurons compared to creWT indicating CaSR regulates

$[Ca^{2+}]_o$-dependent excitability but not via NALCN. The gating curves for creWT and creCasr$^{-/-}$ neurons were similarly left-shifted by the $T_{1.1}$ to $T_{0.2}$ switch. In voltage and current clamp experiments we found that activation of VGSCs was the major contributor to the increase in excitability after solution change from $T_{1.1}$ to $T_{0.2}$. We conclude that regulation of VGSC gating by $[Ca^{2+}]_o$ is the key mechanism mediating $[Ca^{2+}]_o$-dependent changes in intrinsic neocortical neuron excitability. CaSR does not appear to regulate NALCN in neocortical neurons but influences neuronal excitability by its effects on VGSC gating. The creCasr$^{-/-}$ mice were kindly provided by Dr. Wenhan Chang, UCSF and San Francisco VAMC.

Disclosures: B.J. Knight: None. **S.M. Smith:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.17/C32

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant NS050434
NIH Grant MH086400
NIH Grant T32NS062443
Simons Foundation Pilot Award

Title: Ectopic spiking in parvalbumin-expressing inhibitory interneurons of the neocortex

Authors: *B. B. THEYEL;
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Abstract: Action potentials can propagate along axons in both orthodromic and antidromic directions (e.g. *Brain Res Rev.* 21:42, 1995). While spikes are typically initiated in axon initial segments and propagate orthodromically, under various conditions spikes can also be initiated ectopically, from distal regions of axons including their terminals. Among inhibitory interneurons of the hippocampus, ectopic spiking has been observed primarily in NPY-expressing cells, whereas few parvalbumin-expressing interneurons (PV+ cells) generated ectopic spikes (*Nat Neurosci.* 14:200, 2011). We have observed that a large majority (96%) of PV+ cells (which had a “fast-spiking” phenotype) in both orbitofrontal and somatosensory areas of neocortex generated ectopic action potentials after they were sufficiently stimulated. PV+ cells displayed varying patterns of ectopic spiking that could last up to tens of seconds. To initiate ectopic spiking in PV+ cells, we triggered spikes by injecting current steps of increasing amplitude over the course of minutes into the soma. On average, it took 1,236 +/- 772 action potentials (median 989) to elicit the first ectopic action potential. Cells often (~75% of the time) fired trains of ectopic action potentials rather than just one or a handful. Ectopic spikes often

arose during a low-frequency persistent membrane depolarization that lasted a few to hundreds of milliseconds after the current pulse injection, suggesting that in at least some cases we may have recorded a slow depolarization in the terminals that relates to ectopic spike generation. The amplitudes of ectopic spikes varied; amplitudes either exceeded that of somatically triggered spikes or were much smaller (on the order of 3-7 mV) suggesting either a nodal or branch-point failure en route to the cell body from a distal axonal site. Considering the strong inhibition PV+ cells mediate onto excitatory cells and their role in generating cortical gamma rhythms, ectopic spiking may have significant implications for local network activity and cognitive processing. We also observed that most somatostatin-expressing interneurons and even a fraction of pyramidal cells could be induced to generate ectopic spikes, but compared to PV+ cells they did so much less robustly and required more intense stimulation.

Disclosures: B.B. Theyel: None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.18/C33

Topic: B.08. Intrinsic Membrane Properties

Support: NIH (OT2-OD025340)
Fulbright Canada (15122811)
NSERC (PGS M-425353-2012 and PGS D3-437918-2013)
Duke University (University Scholars Program, James B. Duke Fellowship, and Pratt School of Engineering Faculty Discretionary Fund)

Title: *In vivo* quantification of excitation and kilohertz frequency block of the rat vagus nerve

Authors: *N. A. PELOT, W. M. GRILL;
Duke Univ., Durham, NC

Abstract: There is growing interest in treating diseases through electrical stimulation and block of the peripheral autonomic nervous system. Given the widespread connections of the vagus nerve (VN) from the brainstem to most truncal organs, applications include treating epilepsy, depression, obesity, heart failure, and rheumatoid arthritis. Development of and setting parameters for these neuromodulation therapies require understanding the excitation properties of small myelinated and unmyelinated autonomic fibers. We quantified the strength-duration properties, activity-dependent slowing (ADS), and responses to kilohertz frequency (KHF) signals of rapidly conducting (>2 m/s) and slowly conducting (<2 m/s) fibers in the rat vagus nerve.
We stimulated the cervical VN (cVN) and recorded compound action potential (CAP) input-

output curves from the abdominal VN (aVN), from which we quantified the rheobase and chronaxie for fast (μ ± SD = 42 ± 21 μA; 190 ± 34 μs) and slow (96 ± 38 μA; 222 ± 61 μs) vagal fibers.
ADS describes the slowed conduction in peripheral axons resulting from a low frequency pulse train. Three minutes of 2 Hz stimulation slowed the CAP with an increase in latency of 2.3 ± 0.7%, which was approximately constant across fiber conduction speeds.
KHF signals can block neural conduction in peripheral axons. Most experimental studies focused on large efferent fibers and muscle force as the experimental outcome measure. We used CAP recordings to quantify the effects of KHF signals on small autonomic fibers. We found that thresholds were higher to block more slowly conducting fibers and that block thresholds increased monotonically with frequency, in contrast to published findings indicating that block thresholds of unmyelinated axons vary non-monotonically with frequency. Further, there are varied reports on the time for recovery of neural conduction after KHF block; we found that the carryover effect could last tens of seconds following 25 s of KHF signal and that following KHF signals at a sufficiently high amplitude, the neural signal was seemingly not recoverable.
The quantification of mammalian autonomic nerve responses to conventional and kilohertz frequency signals provides essential information for development of bioelectronic medical devices and for understanding mechanisms of action.

Disclosures: **N.A. Pelot:** None. **W.M. Grill:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.19/C34

Topic: B.08. Intrinsic Membrane Properties

Support: Craig H. Neilsen Foundation
NIDDK Fellowship 1 F32 DK116608-01A1

Title: Effects of spinal cord injury on excitability of male mouse. Neurons of the major pelvic ganglion

Authors: *M. L. GRAY[1], D. J. SCHULZ[2];
[1]Univ. of Missouri, Columbia, MO; [2]Div. of Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO

Abstract: The circuit that controls the micturition reflex is composed of autonomic neurons that control smooth bladder muscle (Detrusor) and somatic (voluntary) neurons that control the skeletal muscle of the external urethral sphincter (EUS). After SCI, the coordination between these muscles is lost and they contract spontaneously. While something is known about how SCI

alters the excitability of neuronal pathways that feed into the spinal cord (afferents) and how SCI alters the excitability of these muscles, relatively little is known how excitability of the neurons (autonomic bladder-innervating neurons) contained in a collection of neurons called the major pelvic ganglia (MPG) is altered. Here, we examine the hypothesis that the intrinsic properties of efferent neurons of the pelvic ganglia are altered by chronic spinal cord injury. Animals were injured through laminectomy or transections of T8 of the spinal cord, were examined at either acute (3 days) or chronic (30 days) time points. Neurons of these ganglia were examined for changes in passive, excitability, or action potential properties. While animals undergoing this procedure underwent wasting and had severe increases in post-mortem residual bladder volume, the effects on neurons of the MPG were manifested as relatively modest decreases in excitability. At the population level, the proportion of neurons able to fire 3 or more spikes, and therefore manifesting a so-called 'tonic' phenotype, appeared to be reduced in chronic (but not acute) spinal cord injured animals relative to laminectomy. While input resistance was unaltered, chronic SCI counterintuitively reduced membrane time constant without altering membrane capacitance. Consistent with reduced excitability of chronic SCI, an increase in rheobase and decrease in spike peak amplitude were observed, despite no observed changes in input resistance. Our preliminary data suggests that a possible mediator of this reduction in excitability is downregulation of an as of yet unidentified, inactivation-sensitive, inward current that appeared to be reduced in chronic SCI relative to laminectomy. Together, these data suggest that SCI may reduce excitability in the MPG of the male mouse by a reduction of an inward current.

Disclosures: M.L. Gray: None. **D.J. Schulz:** None.

Poster

040. Neuronal Firing Properties: Modulation, Development, and Pathologies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 040.20/C35

Topic: B.08. Intrinsic Membrane Properties

Support: NSF Graduate Research Fellowship, DGE-1448072
NIH R01 DC004450

Title: Activation of SK channels by spontaneous Ca^{2+} release at dendritic branchpoints of layer 5 cortical pyramidal neurons

Authors: *D. M. ZEPPENFELD[1], L. O. TRUSSELL[2];
[1]Oregon Hlth. Sci. Univ., Portland, OR; [2]Oregon Hearing Res. Ctr., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Spontaneous miniature outward currents (SMOCs) are discrete, Ca^{2+}-activated K^+ channel events that have been reported in diverse populations of neurons across multiple species

including: rat substantia nigra and nucleus basalis of Meynert, salamander retinal ganglion neurons, newt medullary reticular formation, and mouse auditory brainstem neurons. Despite longstanding recognition of these events, there is no established unifying principle to their function. Using whole-cell voltage-clamp recordings and two-photon Ca^{2+}imaging, we report SMOCs localized to dendritic branch points of layer 5 cortical pyramidal neurons. These events were apparent in the presence of TTX and synaptic blockers in juvenile but not adult neurons, and were blocked by TEA and apamin but not iberiotoxin, indicating that they were mediated by SK channels. Ryanodine, but not heparin, reduced SMOC frequency and amplitude, suggesting that SK channels were activated by Ca^{2+}-induced Ca^{2+} release from ER stores. SMOC frequency was reduced in low- Ca^{2+} solution, inhibited by the nonspecific voltage-gated Ca^{2+} channel blocker Cd^{2+}, and completely blocked by the R-type voltage-gated Ca^{2+}channel antagonist SNX-482. While SMOC-mediated membrane hyperpolarizations were evident in current-clamp mode, SMOC blockade did not have a significant effect on action potential shape and had only a modest effect on spike afterhyperpolarization, suggesting that proteins involved in SMOC mediation are not compartmentalized within the soma or axon initial segment. To localize the origin of the Ca^{2+} source that led to the SMOCs, Oregon Green BAPTA 488 was included in the recording pipette for two-photon calcium imaging. We found depolarization-induced Ca^{2+}transients at dendritic branch points that were temporally coincident with a subset of recorded SMOCs. To determine whether SMOCs are a common feature of juvenile mouse neurons, we recorded from diverse juvenile neuron populations and detected them in cerebellar Purkinje neurons, CA1 pyramidal neurons, inferior olive neurons, and dorsal cochlear nucleus principal neurons. These results together with previous studies now suggest that SMOCs are a common feature of juvenile central neurons. Their specific localization to dendritic branch points in layer 5 cortical pyramidal neurons suggests these events may serve to electrically isolate dendritic segments. For example, active, depolarization-induced electrical compartmentalization may serve to limit back propagation of action potentials into dendritic spines or act as a low-pass filter for incoming excitatory potentials.

Disclosures: **D.M. Zeppenfeld:** None. **L.O. Trussell:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.01/C36

Topic: B.10. Epilepsy

Support: FAPESP Grant 2015/22327-7
Fapesp Grant 2016/01607-4
INCT-Translational Medicine 2007/50261-4

Title: Auditory brainstem responses, acoustic startle reflex and prepulse inhibition as markers of susceptibility of audiogenic seizures in rats

Authors: *A. O. CUNHA[1], M. MORADI[4], J. L. DE DEUS[2], C. C. CEBALLOS[7], P. C. G. BARCELOS[5], J. C. OLIVEIRA[6], N. GARCIA-CAIRASCO[8], R. M. LEAO[3];
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Abstract: While acute audiogenic seizures in response to acoustic stimulus appear as an alteration in sensory-motor integration in the brainstem, the repetition of the stimulus leads to the spread of epileptic activity to limbic structures. Here, we investigated whether Wistar Audiogenic Rats (WAR) would have alterations in their auditory response, assessed by the auditory brainstem responses (ABR) and in their sensory-motor gating, which could be related to their susceptibility to audiogenic seizures or to seizure severity. We did not find differences between the amplitudes and latencies of ABR waves in response to clicks for WARs compared to Wistars. Auditory gain and symmetry between ears were also similar. However, hearing thresholds in response to some tones were lower and amplitudes of wave II were larger in WARs. WARs had smaller acoustic startle reflex amplitudes and the percentages of startle inhibited by an acoustic prepulse were higher for WARs than for Wistars. However, no correlation was found between these alterations and midbrain seizure severity or limbic seizure frequency during audiogenic kindling. Our data show that while WARs present moderate alterations in primary auditory processing, the sensory motor gating measured in startle/PPI tests appears to be more drastically altered. We believe that these changes could be correlated with audiogenic seizure susceptibility but not severity.

Disclosures: A.O. Cunha: None. **M. Moradi:** None. **J.L. De Deus:** None. **C.C. Ceballos:** None. **P.C.G. Barcelos:** None. **J.C. Oliveira:** None. **N. Garcia-Cairasco:** None. **R.M. Leao:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.02/C37

Topic: B.10. Epilepsy

Support: NIH/NINDS Grant (NS099007).

Title: Sex differences and the relationship of estrus cycle in a rat epilepsy model of diisoflourophosphate (DFP)-induced neurotoxicity

Authors: *M. C. GAGE[1], M. PUTRA[1], S. SHARMA[2], M. GOLDEN[3], T. THIPPESWAMY[2]; [1]Dept. of Neurosci., [2]Dept. of Biomed. Sci., [3]Dept. of Animal Sci., Iowa State Univ., Ames, IA

Abstract: Research, especially involving rodents, has traditionally excluded female animals from studies. Although variability due to hormone fluctuation does exist, it is of great importance to consider females in any study and include sex as a biological variable (SABV). Sex differences in diseases like epilepsy are highly inconsistent, likely due to the variability between types and severity of epilepsy. We used organophosphate (OP) nerve agent, diisoflourophosphate (DFP), which acts via inhibition of acetylcholinesterase, to induce neurotoxicity. OP nerve agents have historically been used in chemical warfare attacks. Although sex differences, as well as the impact of the hormonal changes during estrus cycle, have been reported in other epilepsy rodent models, there is little evidence of its impact on the OP-induced neurotoxicity. In order to test sex differences and the impact of stages of estrus cycle on *status epilepticus* (SE) and the development of spontaneous recurrent seizures (SRS), we first surgically implanted radiometric telemetry devices in adult male and female Sprague-Dawley rats. Rats were then challenged with 4mg/kgDFP followed by atropine sulfate (ATS) and 2-PAM to reduce mortality. SE as well as epileptogenesis were monitored throughout the course of the experiment for one to three months via continuous video-EEG. We found that females had significantly less seizure severity in comparison to males that were acutely exposed to DFP; this was accompanied by decreased epileptiform spiking and SRS occurrence. Four of eight males developed SRS while only two of seven females developed SRS. Immunohistochemistry of brain sections in females, overall, had significantly less reactive microgliosis compared to males which is possibly due to decreased initial SE severity. Interestingly, the estrus stages had no impact on seizure susceptibility during SE, however, the rats with a greater number of minutes spent in convulsive seizures during SE had a significantly prolonged period of diestrus prior to a regular cycling pattern. We also evaluated a previously demonstrated disease-modifying agent, an inducible nitric oxide synthase inhibitor, 1400W in both male and female rats. Two of seven females treated with 1400W developed SRS while none of the males developed SRS within the first month of DFP exposure. These differences demonstrate the importance of including females while evaluating neurotoxicity and potential disease-modifying agents. As SE severity influences the development of epileptogenesis, and females demonstrate significantly less SE severity with the same dose, SABV should be factored in the design of future OP nerve agent experiments.

Disclosures: M.C. Gage: None. **M. Putra:** None. **S. Sharma:** None. **M. Golden:** None. **T. Thippeswamy:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.03/C38

Topic: B.10. Epilepsy

Support: Conacyt-Mexico (fellowship No. 249772 to DMAC)

Title: Quantitative spatiotemporal analysis of microglia morphology in the developing rat hippocampus after status epilepticus

Authors: *M.-L. LOPEZ-MERAZ[1], D.-M. ALVAREZ-CRODA[2], L. BELTRAN-PARRAZAL[3], C. MORGADO-VALLE[4], D. J. LOANE[5];
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Abstract: Microglia contribution to *status epilepticus* (SE)-induced neuronal injury in the immature brain has not been fully characterized. The goal of this study was to determine the spatiotemporal changes in the microglia morphology in the hippocampus after SE-induced neuronal damage in the infant rat. Thirteen-days-old (P13) male and female rat pups, were given an intraperitoneal injection of lithium chloride (3mEq/kg) and SE was induced 20 h later at P14 by subcutaneous injection of pilocarpine hydrochloride (100mg/kg); control rats were injected with an equal volume of saline. Presence of microglial cells was determined in the CA1 area (*stratum oriens*, SO; *stratum pyramidale*, SP; *stratum radiatum*, SR; and *stratum lacunosum moleculare*, SLM) and the dentate gyrus (molecular layer, ML; granular layer, GL and hilus, Hi) by immunohistochemistry against Iba1. Quantitative analysis of microglia was performed by unbiased stereology 2, 6, 24 and 48 h following SE or control conditions and was correlated to the appearance of neuronal injury detected by fluoro-jade C (F-JC) staining. Microglia morphology characterization considered 4 stages from not activated to activated as follows: ramified, hypertrophic, bushy and amoeboid. The total number of microglia increased in the SP 48 h after SE, as well as in the GL 24 and 48 h after SE when compared with the control group. The morphological analysis showed that 99% of microglia in control conditions had ramified morphology; therefore, comparisons were performed only between SE groups. The number of bushy microglia increased in SP at 6 and 24 h following SE, whereas amoeboid microglia augmented only 48 h after SE. Similarly, bushy microglia increased 24 h after SE in SR. No additional differences were observed in other CA1 strata after seizures. There was an increase in the number of bushy microglia and a decreased in the number of ramified microglia in the ML 6 h following SE. In GL, the number of bushy microglia increased at 6 and 24 h after SE, whereas the amoeboid microglia increased only at 24 h after seizures. Conversely, in this region the ramified microglia decreased 6 and 24 h after SE. In the Hi, the number of bushy and amoeboid microglia increased at 6 and 24 h after SE, while a decrease in the hypertrophic microglia was detected at the same times. Neuronal injury was observed mainly in SP 24 and 48 h after SE and in GL 6 and 24 h following SE. These data demonstrate that SE in the infant rat promotes microglia activation which is associated with the neuronal injury in the hippocampus.

Disclosures: M. Lopez-Meraz: None. **D. Alvarez-Croda:** None. **L. Beltran-Parrazal:** None. **C. Morgado-Valle:** None. **D.J. Loane:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.04/C39

Topic: B.10. Epilepsy

Support: DTRA-JSTO.

Title: Soman-induced cell death and neuroinflammatory response in human acetylcholinesterase knock-in serum carboxylesterase knockout mice

Authors: M. F. STONE[1], **E. R. KUNDRICK**[1], B. M. MARRERO-ROSADO[1], C. R. SCHULTZ[1], K. A. WALKER[1], E. M. MATSON[1], S. J. DEBUS[1], M. DE ARAUJO FURTADO[2], C. CADIEUX[1], *L. A. LUMLEY[1];
[1]US Army Med. Res. Insitute of Chem. Def., Aberdeen Proving Ground, MD; [2]Anatomy, Physiol. and Genet., Uniformed Services Univ. of Hlth. Sci., Bethesda, MD

Abstract: Chemical warfare nerve agents (CWNAs) are acetylcholinesterase (AChE) inhibitors that lead to pharmacoresistant *status epilepticus* (SE) and severe neuropathology when treatment is delayed. Some organophosphorus CWNAs such as soman also inhibit carboxylesterase (CaE), which acts as a bioscavenger to reduce the toxicity of soman. Unlike humans, rodents have plasma CaE activity. A novel humanized mouse strain was used in this study in which the gene expressing serum CaE was interrupted and cross-bred with a mouse strain in which the gene expressing AChE was altered to express the amino acid sequence of the human form of the same protein. This AChE KI/Es1 KO (KIKO) mouse strain might better model human soman exposure compared to wildtype rodents. A model of treatment of soman-exposed KIKO mice with standard medical countermeasures (atropine sulfate, an oxime [HI-6] and a benzodiazepine [midazolam]) was implemented to assess adjunct therapies for neuroprotective potential against soman exposure. Methods. Female KIKO mice were exposed subcutaneously (sc) to a seizure-inducing dose (80 μg/kg) of soman and treated with an admix (intraperitoneal; ip) of atropine sulfate (4 mg/kg) and HI-6 (50 mg/kg) 1 min after exposure, and with midazolam (3 mg/kg; sc) 15 min after onset of behavioral seizure. Twenty-four hours after exposure, mice were perfused with phosphate buffered saline followed by 4% paraformaldehyde, and brains were cryoprotected in 20% sucrose for at least 72 h. Brains were sectioned and stained for evaluation of cell death (using Fluorojade B). In addition, morphological changes in cells immunoreactive for ionized calcium-binding adapter molecule (Iba1), indicative of microglia activation, were assessed. Results. Soman-exposed mice treated with midazolam 15 min after seizure had significant Fluorojade B positive cells (cell death) in the hippocampus, amygdala, thalamus, and piriform cortex 24 h after exposure compared to no agent control mice. In addition, activated microglia was observed in these brain regions 24 h following GD exposure. Conclusions. This

study demonstrates in a novel humanized mouse model that midazolam shortly after onset of toxic signs is not fully protective against the GD-induced neuropathology and neuroinflammatory responses, exemplifying the need for adjunct antiepileptic treatment, such as the NMDA antagonist ketamine to target the glutamatergic component.

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Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.05/C40

Topic: B.10. Epilepsy

Support: National Key Research and Development Program of China: 2017YFC0107200
The Chinese Scholarship Council

Title: Neurovascular coupling during acute interictal events in awake mouse

Authors: *J. LI[1,3], F. YANG[1,3], Y. SONG[5], M. ZHAO[1,2], D. LI[4], J. NIEMEYER[1], W. LIN[3], H. MA[1,2,3,6], T. H. SCHWARTZ[1,2];
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Abstract: Neurovascular coupling based technologies (e.g. fMRI and SPECT) are often used to estimate the spatial distribution of epileptic activity in clinical practice. However, the mechanisms of neurovascular coupling during pathological epilepsy are largely unknown. Previous data were mainly obtained on anesthetized animals little data exist in awake models. We investigated the epileptic neurovascular coupling using calcium imaging to generate spatial maps of neuronal activity and intrinsic optical spectroscopy to measure oxy-hemoglobin (Hbo), deoxy-hemoglobin (Hbr) and total hemoglobin (Hbt), in vivo during interictal spikes (IIS) in awake mouse neocortex to examine their correlations. Seven transgenic mice expressing GCaMP6f in subset of excitatory neurons (Jackson Lab, #024276) were employed in the study. A 5x7mm craniotomy window was created over both hemisphere and the window was sealed with a clear silicone-based polydimethylsiloxane (PDMS) film for long term imaging. Three weeks after the surgery, the mice will be fixed on a clamp for imaging. 5mM bicuculline (0.5ul) was injected in the neocortex to induce interictal spikes. We used simultaneous calcium (illumination at 470 nm) and intrinsic signal at 530 nm and 610 nm imaging to record the neuronal and hemodynamic changes. The IIS showed a monophasic increase in calcium

concentration peaking at 98±24 ms with an amplitude of 159±60% dF/F. Hbr and Hbt also showed monophasic waveform but profound delay, with the peak times of 1076±156 ms (Hbt) and 1364±169 ms (Hbo) and amplitudes of 1.24±0.62 μM (Hbt) and 1.53±0.82 μM (Hbo). The Hbr, on the contrary, showed a bi-phasic waveform, with an early increase (initial dip) peaking at 414±114 ms, with an amplitude of -0.77±0.47 μM, and a late decrease (overshoot) peaking at 1963±154 ms, with an amplitude of 0.65±0.24 μM. Interestingly, we found that the amplitude of Hbr and Hbt during individual IIS was linearly correlated with the interval before IIS (F test, $p<0.01$). The maximal calcium area detected with Chen-Bee method (20% of maximal amplitude) was 6.10±2.25 mm^2. The maximal hemodynamic signals showed bi-lateral distribution with the area of 11.92±3.71 mm^2, 14.95±5.74 $mm^2$and 22.60±6.56 mm^2 for Hbt, Hbo and Hbr dip, respectively. Although hemodynamic signals overestimated the neuronal activity, a high correlation could be found at specific time points in the evolution and dissolution of the hemodynamic signals. Our data suggest that the maximal hemodynamic changes overestimate the neuronal activity during IIS in awake mice. However, hemodynamic signals can better define the spatial extent of excitatory IIS activity at specific time points in their evolution.

Disclosures: **J. Li:** None. **F. Yang:** None. **Y. Song:** None. **M. Zhao:** None. **D. Li:** None. **J. Niemeyer:** None. **W. Lin:** None. **H. Ma:** None. **T.H. Schwartz:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.06/C41

Topic: B.10. Epilepsy

Title: A marmoset model of primary epilepsy

Authors: *X. YANG, Z. CHEN, W. LI;
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Abstract: Primary epilepsy is a chronic recurrent syndrome of transient nervous system dysfunction. It can not only lower the quality of patients' life, but also cause serious social load. The pathogenesis of primary epilepsy is complicated and its mechanism remains unclear. One reason is that there is no suitable model animal for clinical translational research. In the course of daily feeding, we found similar behavioral phenotype of primary epilepsy existed in a specific population of marmoset (*Callithrix jacchus*). However, the epilepsy phenotype is undefined. In this study, we aim to identify the epileptic phenotype from both gene and behavioral levels. Our results showed that the epilepsy marmoset had gene mutation and clear behavioral phenotype of epilepsy. To gain phenotypic and mechanistic insights, we employed scalp EEG and electrocorticogram to identify epileptiform discharges under anesthesia and free moving

condition. Our results specified the epilepsy phenotype in the marmoset family and provide a potential marmoset model of primary epilepsy for pathogenesis and drug research.

Disclosures: **X. Yang:** None. **W. Li:** None. **Z. Chen:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.07/C42

Topic: B.10. Epilepsy

Title: Anticonvulsant drug actions in a rat model of birth asphyxia

Authors: M. JOHNE[1,2], K. RÖMERMANN[1], P. HAMPEL[1,2], W. THEILMANN[1], T. ALA-KURIKKA[3], *K. KAILA[3], W. LÖSCHER[1,2];
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Abstract: Seizures are the most frequent type of neurological emergencies in newborn infants during the first 28 days of life.Neonatal seizures occur following birth asphyxia, CNS infections or intracranial hemorrhage. Phenobarbital (PB) is currently the most widely used anticonvulsant drug for treatment of neonatal seizures, but it fails to stop them in ~50% of cases. In a preclinical rat trial, bumetanide, an inhibitor of the Na-K-2Cl cotransporter NKCC1, augmented the effects of PB in a hypoxia model of neonatal seizures (Cleary et al., 2013) whereas it was ineffective in a human trial (Pressler et al., 2015). The aim of this study was to evaluate the effects of PB, midazolam (MDZ) and bumetanide on seizures in a novel model of perinatal, intermittent asphyxia in rats (Kaila et al., in preparation) designed for better translation to the human situation. In 11-day-old rats, intermittent asphyxia was induced by an increase in CO_2 (20% of atmospheric pressure) with stepwise changes in O_2 (5-9%) applied for 30 min at constant ambient temperature (35.5 °C). After the asphyxia, animals were promptly re-exposed to room air. MDZ (1 mg/kg), bumetanide (0.3 mg/kg), PB (15 or 30 mg/kg) or bumetanide+PB (0.3+15 mg/kg) were administered i.p. 0, 15, 15 or 15+30 min before asphyxia, respectively. In an additional trial, we injected PB (30 mg/kg) or MDZ (1 mg/kg) i.p. directly after asphyxia. All untreated animals developed seizures within 10 minutes after the end of asphyxia. 30 mg/kg PB or MDZ given before the asphyxia decreased the seizure incidence to 22% or 20%, respectively. MDZ retained its effect when it was given after asphyxia. None of the other treatments had an effect on seizure occurrence. These data show that bumetanide does not increase the efficacy of phenobarbital on neonatal seizures in our asphyxia model, consistent with the human trial by Pressler et al. (2015). Our study shows that the present asphyxia model is a useful tool for

evaluating the efficacy of drugs used to, or intended to, suppresses neonatal seizures. Unlike rodent hypoxia models, the present asphyxia model enables the examination of anticonvulsant drug actions *after* the hypoxia/asphyxia conditions, which is important for translation to the human situation.

Disclosures: K. Kaila: None. **M. Johne:** None. **K. Römermann:** None. **P. Hampel:** None. **W. Löscher:** None. **T. Ala-Kurikka:** None. **W. Theilmann:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.08/C43

Topic: B.10. Epilepsy

Support: NINDS U01 NS090340
NINDS NS29709
NINDS F32 NS105329

Title: A genetic cross between Gad2-Cre and loxTB Htr2c mouse strains does not prevent seizures and death in a 5-HT_{2C}-null SUDEP mouse model

Authors: *C. A. MASSEY, J. L. NOEBELS;
Neurol., Baylor Col. of Med., Houston, TX

Abstract: Sudden Unexpected Death in Epilepsy (SUDEP) accounts for up to 17% of all deaths in individuals with epilepsy. The pathophysiological mechanisms underlying SUDEP remain unclear, but data from human and animal studies suggest that cardiorespiratory dysfunction following a generalized tonic-clonic seizure (GTCS) may play an important role.
We previously reported a novel adult-onset SUDEP mouse model (loxTB Htr2c) with disrupted transcription of the X-linked serotonin 2C receptor (5-HT_{2C}R). These mice display a complex epileptic phenotype and increased premature mortality. 5-HT_{2C}Rs are found throughout the brain, and previous reports indicate that many of the cells that express 5-HT_{2C}Rs are GABAergic neurons. We hypothesized that re-expressing 5-HT_{2C}Rs in GABAergic neurons would eliminate seizures and premature death in loxTB Htr2c mice. Crossing loxTB Htr2c mice with a strain expressing Cre recombinase excises the transcription blocker cassette in the *Htr2c* gene and allows for expression of 5-HT_{2C}R in a Cre-dependent manner (Xu et al., 2008). We bred the loxTB Htr2c strain with mice that express Cre recombinase in Gad2-positive cells ($Gad2^{Cre/Cre}$). Offspring from this crossing have the transcription blocker excised in Gad2-positive cells. We then used video-EEG recordings to determine if the mice still displayed seizures and SUDEP. We found that loxTB Htr2c and heterozygous Gad2-Cre ($5\text{-}HT_{2C}^{loxTB/Y}/Gad2^{Cre/+}$) male mice had a complex epileptic phenotype that recapitulated our previous data for 5-HT_{2C}-null mutants,

including spike-wave discharges similar to some absence epileptic mouse models and behaviorally silent generalized nonconvulsive seizures. These mice also exhibited GTCS and in one mouse we recorded a SUDEP event. The survival of these mice did not improve compared to 5-HT_{2C}-null mice. We found that at postnatal day 300 only 62% of male 5-$HT_{2C}^{-/Y}$/Gad2$^{Cre/+}$ mice survived (31/50) compared to 100% of wildtype (5-$HT^{+/Y}$/Gad2$^{Cre/+}$) littermates (39/39). This mortality profile is similar to the 5-HT_{2C}-null mice we previously reported where only 65.66% of 5-HT_{2C} mice survive (65/99).
These data suggest that Gad2-Cre directed expression of 5-HT_{2C}R is not sufficient to prevent seizures and premature death in a 5-HT_{2C} SUDEP model. Ongoing experiments are validating the precise levels of 5-HT_{2C}R re-expression in Gad2-positive GABAergic neurons in these mice. Since it is possible that the mice need two copies of Gad2-Cre to attain normal expression of 5-HT_{2C}R, we have adjusted our breeding scheme to obtain homozygous Gad2-Cre mice. Future experiments will continue to investigate the mechanisms that underlie epilepsy and SUDEP in 5-HT_{2C} mutant mice.

Disclosures: C.A. Massey: None. **J.L. Noebels:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.09/C44

Topic: B.10. Epilepsy

Title: Optimization of a zebrafish epilepsy model for testing anti-epileptic drugs

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Abstract: Epilepsy is a brain disorder that produces recurrent seizure activity. In severe cases, seizures can cause developmental delays and long-term cognitive impairment. Thirty percent of patients with epilepsy are non-responsive to anti-epileptic drugs (AED). To address this problem, improved AED screening methods are needed. Previous studies showed that the zebrafish is a potentially useful and effective model organism for high throughput screening of current and novel AEDs. These studies used pentylenetetrazole (PTZ) to induce seizures. Previous PTZ zebrafish assays observed a time frame of 30 minutes and used a concentration of 20 mM PTZ (Afrikanova et al. 2013, Gupta et al. 2018). A 30-minute observation window hinders the ability to fully assess AED effects on seizure activity. In this study, we optimized the PTZ assay by maintaining a consistent level of seizure activity over 90 minutes. Seizure activity was maintained by using 10 mM PTZ, instead of 20 mM PTZ, and was based on the number of large

movements (any movement at speed >8mm/sec). The Viewpoint Zebrafish box was used to quantify zebrafish behavior and movements, which provides precise measurement of AED efficacy. In order to asses AED efficacy, we pretreated one group with AEDs prior to PTZ exposure and a second group was treated at the same moment of PTZ exposure. These changes to the previous PTZ kindling models provide a more sensitive measurement of AED efficacy on seizure responsiveness. In our optimized assay, we showed that carbamazepine and topiramate reduced seizure activity, while lamotrigine and GS967 increased seizure activity. These results differ from what has been previously established by other PTZ-induced models. Overall, these experiments showed that using 10 mM PTZ allows for a comprehensive method to evaluate AED efficacy, along with the two different methods of pretreating and non-pretreating the zebrafish with AED. These experiments also lay the groundwork for testing the efficacy of AEDs on genetic epilepsy models.

Disclosures: P. Milder: None. **J. Marrs:** None. **T.R. Cummins:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.10/C45

Topic: B.10. Epilepsy

Support: Grant MOP-102599
RGPIN 2015-05103

Title: A rat model of somatosensory-evoked reflex seizures induced by peripheral stimulation

Authors: *A. BORTEL, Z. YAO, A. SHMUEL;
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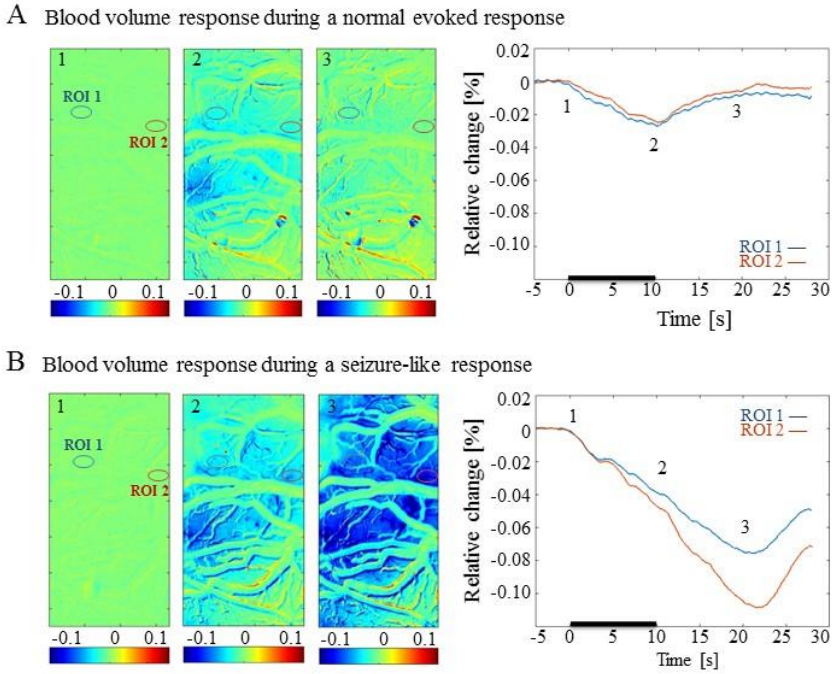
Abstract: Methods for eliciting epilepsy in animal models often induce seizures in large parts of the brain or damage the brain. We introduce an animal model of somatosensory-evoked reflex seizures which generates focal seizures without causing damage to the brain.
We performed all experiments in adult, 100-107 days old male Sprague-Dawley rats. Specifically, we electrically stimulated digits or forepaws of rats sedated with dexmedetomidine while imaging cerebral blood volume and recording neurophysiological activity in cortical area S1FL. For the recordings, we either inserted a linear probe into the D3 digit representation or we recorded from a surface electrocorticography (ECoG) array placed above the dura mater. Peripheral stimulation elicited either normal evoked responses or high-amplitude seizure like responses. Seizures generated during the stimulation period showed prolonged discharges following the cessation of the stimulus. High-frequency oscillations were observed prior to and during the seizures, with amplitudes higher than those associated with normal evoked responses.

Seizures were typically followed by refractory periods. Optical imaging of cerebral blood volume showed that the seizures were initially focal and propagated from the onset zone to a larger territory, during the stimulation and following its cessation (Fig. 1). Seizures were recorded not only by probes inserted into cortex but also with ECoG arrays placed over the dura mater, indicating that the seizures were not induced by damage caused by inserting the probes to cortex. Stimulation of the forepaw elicited more seizures than stimulation of a digit. Unlike rats sedated with dexmedetomidine, rats anesthetized with urethane showed no ictal discharges, indicating that the seizures depended on the dexmedetomidine.
Our proposed animal model generates seizures evoked by electrical sensory stimulation free of artifacts and brain damage. It can be used for studying the laminar-specific mechanisms underlying the generation and propagation of reflex seizures and for evaluating antiepileptic drugs.

Cerebral blood volume response evoked by digit stimulation.



A. Cerebral blood volume response evoked by stimulation of a digit (D3). To the left, the spatial responses before (1s before), during (9-10s), and after (19-20s) the 10s-long digit stimulus onset. The reference for obtaining these responses was imaged between 3 and 1 seconds before stimulus onset. Note that negative response indicated in indexed blue color represent increase in blood volume. To the right are two time-courses presenting the corresponding temporal response from two regions (blue and red ROIs) within the activated area. The stimulation period between 0 and 10 seconds is marked by a dark bar. **B.** Maps of the blood volume changes during seizure-like response from before, during, and after the 10s-long digit stimulation period (exact time periods are as in A). To the right are two time-courses presenting the corresponding temporal response from two regions (blue and red ROIs) within the activated area.

Disclosures: A. Bortel: None. **Z. Yao:** None. **A. Shmuel:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.11/C46

Topic: B.10. Epilepsy

Support: National Key Research and Develpment Program of China:2017YFC0107200
The Chinese Scholafrship Council

Title: The spatiotemporal dynamic of neuronal and hemodynamic changes during acute ictal events in awake mouse

Authors: *F. YANG[1,3], J. LI[1,3], Y. SONG[5], M. ZHAO[1,2], J. NIEMEYER[1], D. LI[4], W. LIN[3], H. MA[1,2,3,6], T. H. SCHWARTZ[1,2];
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Abstract: Identifying the epilepsy focus with high spatial resolution is a challenge in clinical practice, especially in the cases where no anatomical abnormality can be detected. Neurovascular coupling based functional brain technology (e.g. fMRI, SPECT) is often applied in clinic but the neurovascular coupling mechanism during ictal events is still unclear. Our lab previously demonstrated that the cerebral volume increased co-localized with neuronal activity in anesthetized model. In this study, we investigate the neurovascular coupling mechanism during acute ictal events in unanesthetized mice. Seven transgenic mice expressing GCaMP6f in subset of excitatory neurons were employed in the study. A 5x7mm craniotomy window was created over both hemispheres and the window was sealed with a clear silicone-based polydimethylsiloxane (PDMS) film for long term imaging. Three weeks after the surgery, the mice will be fixed on a clamp for imaging.2mM 4AP (0.5ul) was injected in the neocortex to induce ictal events. We used simultaneous calcium (illumination at 470 nm) and intrinsic signal at 530 nm and 610 nm imaging to record the neuronal and hemodynamic changes during ictal events. The ictal event contained a train of spikes. Each spike showed a propagation wave of calcium increase. The spatial extent of each spike gradually increased over time. In some cases, the ictal event propagated to the contralateral cortex. Different propagation patterns could be recorded when crossing the hemisphere. In some ictal events, the brain area close to the midline in the contralateral hemisphere became involved first, showing a smooth propagation rather than a jump across the corpus callosum (CC). In some events, the brain area mirroring the 4AP injection site involved the ictal events first, showing CC jumping propagation to a mirror focus. The total hemoglobin (Hbt) showed an increase with ictal events. The spatial extent of Hbt change closely resembled the spatial spread of calcium signal. The oxy-hemoglobin changes also

showed an increase with ictal events, but spatially overestimated the calcium activity. The deoxy-hemoglobin (Hbr) signal showed complex spatiotemporal dynamics. In the surrounding area, a decrease in Hbr was observed throughout the seizure. In the epi-focus, both increase and decrease in Hbr were observed. Our data indicated that cross hemisphere propagation may involve either contiguous spread or cross callosal white matter spread. Neuronal activity induced Hbt changes best represent the spatial involvement of seizure activity, indicating that cerebral blood volume based imaging techniques may be better than BOLD based imaging techniques for seizure mapping.

Disclosures: **F. Yang:** None. **J. Li:** None. **Y. Song:** None. **M. Zhao:** None. **J. Niemeyer:** None. **D. Li:** None. **W. Lin:** None. **H. Ma:** None. **T.H. Schwartz:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.12/C47

Topic: B.10. Epilepsy

Support: Bill and Melinda Gates Foundation Cysticercosis Elimination in Peru grants 23981 and 33848 (H.H.G.)
NIH grant 5D43TW006581 (Infectious Diseases Training Program in Peru) (R.H.G.)
NIH training grant FIC/NIH D43 TW001140, and Fondo para la Innovación, Ciencia y Tecnología FINCyT grant 129-FINCyT-IB-2013 (M.R.V.)
Innovate Perú Nro.135-PNICP-PIAP-2015

Title: Evaluation of neuroinflammation, number and location of cysts in a rat epilepsy model with neurocisticercosis

Authors: *A. D. DELGADO[1], R. C. OROZCO[1], R. H. CELIZ[1], M. VERASTEGUI[1], R. GILMAN[2];
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Abstract: Neurocysticercosis (NCC) is caused by the larva of the taenia solium located in the central nervous system (CNS). In endemic countries, it is the main cause of late epilepsy. Our group has developed a rat model to study the electrophysiology of the waveforms of seizures in neurocysticercosis. In preliminary studies we have observed that our model allows the development of viable cysts in the brain, and the presence of generalized tonic clonic and silent seizures, which allows us to have a model similar than humans. Our objective is to evaluate neuroinflammation- disruption of the blood-brain barrier, number and location of cysts in rats with neurocysticercosis that developed epilepsy. Male Holtzman rats received intracranial

infection with activated T. solium oncospheres between 12-15 days of birth, after 3 months of infection MRI T2 were performed in order to detect the presence of the cysts in the rat brain. Selected Infected rats (n=12) and not infected rats (n=8), were continuously recorded by video telemetric electroencephalography (tEEG) to monitor the brain activity and detect seizures for four weeks. Abnormal hypersynchrony of neuronal activity was observed in the tEEG recording associated with seizures in 33% (n = 4), with an average duration of 120 seconds per seizure. These rats had the highest number of parenchymal cysts in cerebral cortex; immunohistochemistry studies were performed to observe neuroinflammation and disruption of the blood-brain barrier.

Disclosures: A.D. Delgado: None. **R.C. Orozco:** None. **R.H. Celiz:** None. **M. Verastegui:** None. **R. Gilman:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.13/C48

Topic: B.10. Epilepsy

Support: NIH/NINDS R01-NS096976
NIH/NINDS R01-NS103139

Title: Modeling catastrophic childhood genetic epilepsies: The epilepsy zebrafish project (EZP)

Authors: *C. A. CARPENTER[1], J. LIU[1], A. GRIFFIN[1], B. GRONE[2], K. HAMLING[3], M. T. DINDAY[1], M. ANVAR[1], C. ONONUJU[4], R. PATERNO[1], S. C. BARABAN[1];
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Abstract: Pediatric catastrophic epilepsies lead to medically intractable early-life seizures. Patients also tend to develop debilitating neurodevelopmental, cognitive and behavioral problems. Many of these children are diagnosed with genetic forms of epilepsy and to date almost 70 *de novo* single-gene mutations have been identified in this patient population. Our understanding and treatment of these genetic epilepsies have been limited, in part, by the cost and time needed to model these genes in rodents. Zebrafish (*Danio rerio*) stand as a great alternative model for pediatric epilepsies as they have a high degree of genetic similarity with humans (>80% for disease-causing genes) and provide the opportunity for basic research and large-scale drug discovery efforts. Here, we report the use of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 gene editing to generate zebrafish for catastrophic childhood epilepsies. Regions of similarity between zebrafish and human genes were identified

using BLAST and gene expression levels were assessed between 0 and 7 days post-fertilization (dpf) using qPCR. Zebrafish lines were generated using CRISPR/Cas9 genome editing and raised to F3 or greater. All lines were screened, in a blinded fashion, using survival, behavioral and electrophysiological assays between 3-14 dpf with *post hoc* genotyping. Behavioral and electrophysiological data were imported to MATLAB (Mathworks) for automated analysis and statistical significance was determined using GraphPad Prism 8 software. Loss-of-function zebrafish lines were generated for 38 human epilepsy genes. Here we report that early fatality was noted in three lines. Spontaneous ictal-like seizures, characterized as long-duration, large-amplitude, multi-spike electrographic activity, were confirmed in six lines. Zebrafish mutants for two of these epileptic lines were noted to have abnormal hypoactive behaviors. This work shows that zebrafish can be used to rapidly and efficiently model genetic epilepsies seen in children. This approach allows us to begin to define the functional consequences of monogenic gene mutations *in vivo* and gives us a powerful platform for drug screening and development.

Disclosures: C.A. Carpenter: None. **J. Liu:** None. **A. Griffin:** None. **B. Grone:** None. **K. Hamling:** None. **M.T. Dinday:** None. **M. Anvar:** None. **C. Ononuju:** None. **R. Paterno:** None. **S.C. Baraban:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpyGenix Therapeutics.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.14/C49

Topic: B.10. Epilepsy

Title: Fisetin attenuates behavioural impairment and inflammatory response in pentylenetetrazole-induced kindling model of epilepsy by modulating neuronal plasticity

Authors: *S. KHATOON[1], M. SAMIM[2], N. AGARWAL[3];
[1]Dept. of Med. Elementology and Toxicology, [2]Dept. of Chem., [3]Ctr. for Translational and Clin. Res., Sch. of Chem. and Life Sciences, JAMIA HAMDARD, New Delhi, India

Abstract: Background: Epilepsy is a chronic neurological disease, introduced as the fourth most common neurological disorder affecting about 65 million people worldwide with 90% of them residing in developing countries. About 30% of the patients receiving current antiepileptic drugs remain refractory to the disease owing to symptomatic treatment. This urges the need to find a molecule having molecular interactions. Fully kindled seizures are similar to complex partial seizures with secondary generalization, to the extent that kindling is considered as a model of temporal lobe epilepsy in humans. **Aim:** The present study was designed to evaluate the effect of fisetin (FST) on seizures manifestations, memory performance, inflammatory mediators, synaptic plasticity, neurodegeneration and oxidative stress in pentylenetetrazole

(PTZ)-induced kindling model. **Material and Methods**: Chemical kindling was induced by repetitive i.p. injections of PTZ at subconvulsive doses (25mg/kg) on alternate days in Swiss albino mice for 35 days. Each group consisted of 10 animals : Vehicle (0.1% carboxymethylcellulose, CMC) daily + saline, i.p.; Vehicle (0.1% CMC) daily+ PTZ; FST (5 mg/kg, p.o.) daily+ PTZ; FST (10 mg/kg, p.o.) daily+ PTZ; FST (20 mg/kg, p.o.) daily+ PTZ; Valproic acid (200mg/kg i.p) + PTZ. The behavioral signs of seizures were monitored and were scored as per Racine scale. Step down latency and transfer latency was used to assess spatial learning and memory. Histopathological changes were ascertained in the hippocampus by H & E staining and Nissl staining. Gene analysis and immunostaining was carried out to evaluate the expression of inflammatory mediators and neuronal plasticity markers. The redox status was estimated by measuring lipid peroxidation and protein carbonyl levels in hippocampus tissue. **Result:** The results show that FST suppresses seizure, memory dysfunction, neurodegeneration and free radicals in a dose-dependent manner. However, it was found that the mRNA level and immunoreactivity of inflammatory factors were significantly downregulated in FST treated animals in a dose-dependent manner. Moreover, FST treatment induced the expression of cyclic adenosine monophosphate response element binding protein- brain derived neurotrophic factor (CREB-BDNF). The results were comparable to that of already available antiepileptic drug, valproic acid used as a positive control in the study. **Conclusion:** Based on the results of the present study, we concluded that FST might serve as a therapeutic agent for the treatment of chronic epilepsy.

Disclosures: S. Khatoon: None. **M. Samim:** None. **N. Agarwal:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.15/C50

Topic: B.10. Epilepsy

Support: ANR-16-CE17-0013-01

Title: Silencing the epileptic network in a bilateral sub-cortical band heterotopia rat model

Authors: *D. HARDY, V. PLANTIER, E. BUHLER, A. VINCK, F. WATRIN, A. REPRESA, J.-B. MANENT;
INMED U1249, INSERM Aix-Marseille Univ., Marseille, France

Abstract: Cortical malformations are the most frequent causes of drug-resistant childhood epilepsies. Current treatment options for pediatric epilepsies include antiepileptic drugs and surgical removal of epileptogenic brain tissue but these options remain unadapted to a majority of patients. Recently, a novel preclinical model of an epileptogenic cortical malformation,

obtained by knocking down *in utero* Dcx gene expression in rats, has been developed in our laboratory. These animals display sub-cortical band heterotopia bilaterally and recapitulate several features of a pediatric epilepsy syndrome such as early onset seizures (Sahu et al., 2019) and a disorganization of functional connections (Plantier et al., 2018). The goal of this study is to determine if, in this model, we can efficiently suppress the excitability of the malformation *in situ* and if it can ameliorate epilepsy phenotype and prevent/delay epilepsy onset, in order to modify disease progression or mitigate seizures severity. To address this question, different approaches to achieve constitutive or conditional localized suppression of neuronal excitability in the malformation have been tested. Efficiency of each silencing approach has been first tested with several *in vitro* experiments on acute slices. To determine passive and synaptic cell properties whole-cell patch-clamp recordings were performed. To ascertain if silencing the malformation can affect network connectivity, the strength of excitatory and inhibitory inputs received by cortical pyramidal cells has been investigated with laser scanning glutamate uncaging experiments. Susceptibility to induced seizure of each silencing strategies has been tested on acute slices by recording local field potential in cortical layers following bath application of a $GABA_A$ receptors antagonist. The different silencing approaches were finally tested *in vivo* by recording EEG in rats to determine if epileptic symptoms were ameliorated or not, and which approach was the most efficient.

Disclosures: D. Hardy: None. **V. Plantier:** None. **E. Buhler:** None. **A. Vinck:** None. **F. Watrin:** None. **A. Represa:** None. **J. Manent:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.16/C51

Topic: B.10. Epilepsy

Support: NINDS R01 NS099348
Wellcome Trust Grant 209164/Z/17/Z
Wellcome Trust Grant: 204788/Z/16/Z

Title: Epileptic seizures lead to a loss of near-critical brain organisation in the zebrafish brain

Authors: *D. BURROWS[1], R. E. ROSCH[3], D. S. BASSETT[4], M. P. MEYER[2];
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Abstract: Zebrafish have emerged as an important new model for epilepsy. In part, its utility lies in the relative ease with which genetic disorders (including different epilepsies) can be modelled.

Moreover, recent advances in microscopy now allow fast whole-brain functional calcium imaging at near single-cell resolution, including during epileptic seizures. In line with a variety of other model systems, neuronal activity in the zebrafish brain has recently been found to display markers of criticality, such as powerlaw-like distributions of the size and duration of neuronal avalanches.Here we report volumetric light-sheet, and two-photon recordings of zebrafish expressing the fluorescent calcium indicator GCaMP6s both at rest and during pentylenetetrazole (PTZ)-induced epileptic seizures. We show both changes of single neuron firing properties and changes in the statistics of neuronal avalanches during PTZ exposure. Furthermore, we test whether functional networks during PTZ exposure are enriched for specific topological features - such as strongly connected cycles - that support these longer avalanches.Our findings indicate that the zebrafish brain deviates from criticality during epileptic seizures. Similar observations have previously been made in human recordings, further supporting the validity of zebrafish as a model for epilepsy and epileptic seizures. Because of the spatial resolution this model system affords, we can then link observations of changes in neuronal avalanche statistics to mesoscale topological features recorded at near-single cell resolution. This work illustrates how advanced imaging in zebrafish models of epilepsy may support a more in-depth understanding of multi-scale dynamics of epileptic seizures.

Disclosures: **D. Burrows:** None. **R.E. Rosch:** None. **D.S. Bassett:** None. **M.P. Meyer:** None.

Poster

041. Animal Models of Epilepsy I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 041.17/C52

Topic: B.10. Epilepsy

Title: Fast 2-photon imaging of excitatory and inhibitory subpopulations in PTZ-treated larval zebrafish reveals patterns of initiation and propagation during ictal-like events

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Abstract: Epilepsy is a debilitating neurological disorder characterized by recurring seizures. Unfortunately, the manner in which these seizure events initiate and spread is not fully understood. By examining the fundamental mechanisms of seizure propagation in model organisms we hope to provide a greater understanding of epilepsy as well as uncover potential therapeutic options. While mammalian seizure models have provided insights and treatments for

the disorder, the emerging zebrafish model also provides an opportunity to understand ictal-like events with unprecedented imaging of the entire nervous system. By combining electrophysiology and 2-photon calcium imaging in transgenic GCaMP6f-expressing zebrafish, we determined the patterns by which ictal-like events initiated and propagated across the brain. In our experiments, ictal-like events were induced in larval zebrafish (dpf 4-7) by bath application of the convulsant Pentylenetetrazole (PTZ, 15 mM). Event characterization as well as single-cell resolution of activity across multiple brain areas at varying brain depths was recorded with simultaneous local field potential recording and calcium imaging. Across animals, events initiate in various brain regions and propagate in multiple directions. However, within individuals, events tend to follow similar propagation patterns. Further, across animals we observed weak involvement of the telencephalon in events that otherwise spread across most brain regions. Fast imaging (10-100 Hz) of excitatory and putative inhibitory neurons - differentiated by nuclear pan-neuronal GCaMP6f expression combined with DsRed co-expressed in excitatory VGlut2.1+ neurons - allowed us to examine differences in roles of these cell types in event propagation. Preliminary analyses show that inhibitory neurons in larval zebrafish generally do not lead ictal-like events; in fact, in some events the earliest-active neurons are excitatory. In sum, our findings suggest that PTZ-induced ictal-like patterns in larval zebrafish are unique across, but similar within, individual animals. These findings provide fundamental information about seizure propagation in the zebrafish PTZ model, which has implications for future studies and use of this model in potential drug screening.

Disclosures: J.E. Niemeyer: None. **P. Gadamsetty:** None. **S. Sylvester:** None. **H. Ma:** None. **E. Aksay:** None. **T.H. Schwartz:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.01/C53

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

Support: Weston Brain Institute
CIHR

Title: Evaluating the neuroprotective potential of exosome delivered catalase-SKL in a pre-clinical model of Alzheimer's disease

Authors: *Q. LIU[1], S. HAYES[1], P. KISER[2], S. SELVAKUMARAN[1], B. L. ALLMAN[1], P. WALTON[1], S. N. WHITEHEAD[1];
[1]Anat. and Cell Biol., [2]Pathology and Lab. Med., Schulich Sch. of Med. and Dentistry, Western Univ., London, ON, Canada

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder that accounts for the majority of dementia cases. A core pathological feature of AD is the over-production of reactive oxygen species (ROS) which mediate oxidative stress in the brain. Peroxisomes play a crucial role in mitigating the accumulation of ROS due to the presence of catalase, an anti-oxidant enzyme found within the organelle. As the brain ages, progressive dysfunction of peroxisomes and decreased localization of catalase into the organelle contributes to elevated ROS. A suitable therapy to address the increased accumulation of ROS in AD has yet to be identified. In particular, the use of non-specific antioxidants has been disappointing due to physiological barriers common to human subjects and preclinical AD models. These barriers are the immune reaction to therapeutics and the ability of candidate agents to penetrate the brain's blood-brain barrier. To address these issues, our team has engineered a recombinant derivative of the antioxidant enzyme catalase (CAT-SKL) that specifically targets peroxisomes, thereby providing powerful organelle-based antioxidant and anti-inflammatory effects. In the present study, we aimed to (i) establish the packaging CAT-SKL into macrophage-derived exosomes—endogenous membrane vesicles for transport of proteins between cells—to generate an effective way of targeting our therapeutic to cells undergoing oxidative stress while minimizing degradation in the bloodstream, and (ii) determine the safety and bio-distribution in vivo. CAT-SKL was packaged into macrophage-derived exosomes via sonication, and loaded exosomes were purified using size exclusion chromatography or ultracentrifugation. Successful loading of CAT-SKL was confirmed using western blots and H2O2 decomposition assays. Male and female, wildtype and APP/PS1 transgenic mice were intranasally administered biotinylated CAT-SKL packaged in exosomes and tissues of interest were harvested and processed for fluorescent and immunohistological staining. Bioavailability and off-target toxicity were assessed post mortem. Diffuse labeling of CAT-SKL in the brains of these mice supports the intranasal administration of exosomes as an effective delivery mechanism to bypass issues surrounding bioavailability, antigenicity, and the blood-brain barrier. Histopathological assessments of the brain and off-target tissue demonstrate no toxicity. Future directions will investigate the efficacy of exosome-mediated delivery of the targeted antioxidant CAT-SKL to prevent and/or ameliorate pathological and behavioral outcomes in a transgenic mouse model of AD (APP/PS1).

Disclosures: Q. Liu: None. **S. Hayes:** None. **P. Kiser:** None. **S. Selvakumaran:** None. **B.L. Allman:** None. **P. Walton:** None. **S.N. Whitehead:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.02/C54

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

Title: Preclinical model for Alzheimer's disease: The confluence of aging, genetic risk, and diet

Authors: *B. COLARUSSO[1], P. KULKARNI[1], J. YEBOAH[1], M. GUPTA[1], X. CAI[1], E. KOURANOVA[2], J. HARTNER[3], C. FERRIS[1];
[1]Psychology, Northeastern Univ., Boston, MA; [2]Genome Engin. and iPSC Ctr., Washington Univ., St Louis, MO; [3]Horizon Discovery Ltd, Cambridge, United Kingdom

Abstract: The primary cause of dementia in the elderly population is Alzheimer's disease (AD), with the number of those who currently suffer from the disease hovers around 35 million people worldwide. Symptomology of AD is most prominently described by neurodegeneration in areas of the brain specific to memory, such as the hippocampus. The most significant risk factor for AD is age, with the prevalence of the disease doubling every five years following the threshold of 65 years of age. The second most significant risk factor cited in much of the current literature on the disease is the primary genetic marker, the ε4 allele of the cholesterol transporter apolipoprotein E (ApoE4). Additional risk factors include head injury, obesity and diabetes, insufficient physical exercise, and hypertension, the latter three of which may all coincide, greatly increasing one's risk for AD.
The present study examined the intersection of both aging and diet as risk factors for Alzheimer's disease with wildtype (n = 4 males; n = 5 females) and ApoE4 knock-in (n = 5 males; n = 6 females) rats over three time points throughout the course of their lives while on a "Western Diet." At four months of age, subjects were tested for cognitive behavior and imaged using various MRI modalities including T1 weighted voxel-based morphometry, diffusion-weighted imaging with quantitative anisotropy, resting state and BOLD functional connectivity using a 7.0T scanner. At seven months of age, the animals were placed on a high fat, high fructose diet termed the "Western Diet" to examine its effect on AD progression and global cognitive functioning. Two subsequent behavior and imaging sessions were repeated at 3-4 month intervals after the change in diet before termination of the study. All images were registered to a 3D MRI Rat Atlas with 171 segmented and annotated brain areas used to generate an unbiased computational analysis of all data.
There were significant gender differences in many neuroradiological measures, most surprisingly in the male WT animals which exhibited greatly decreased cognitive abilities during behavior testing. Male animals in both ApoE4 and WT groups also exhibited significant differences in fractional anisotropy as compared to their female counterparts after 8 months on the high fat, high sugar diet. Female connectivity was greatly reduced as compared to normal females at 4 months of age. The male ApoE4 showed hypoconnectivity in areas associated with learning, memory, and emotion.

Disclosures: **B. Colarusso:** None. **P. Kulkarni:** None. **J. Yeboah:** None. **M. Gupta:** None. **X. Cai:** None. **E. Kouranova:** None. **J. Hartner:** Other; Horizon Discovery Ltd provided the animals used in this study. **C. Ferris:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Horizon Discovery provided the animals in the study.. Other; Craig Ferris (PI) has a financial interest in Animal Imaging Research, the company that makes the rat equipment..

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.03/C55

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

Support: NINDS Grant R01 NS062184
NIMH Grant R01 MH096093
Harvey Family Endowment (ELB)

Title: Mutant amyloid precursor protein (APP) increases axonal transport rates in the hippocampal-basal forebrain memory circuit: An MRI and confocal imaging study

Authors: E. L. BEARER[1,2,3], C. S. MEDINA[1], *R. E. JACOBS[4];
[1]Dept. of Pathology, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [2]Biol. and Biol. Engin., Caltech, Pasadena, CA; [3]Marine Biol. Lab., Woods Hole, MA; [4]Zilkha Neurogenetics Inst., USC Keck Sch. of Med., Los Angeles, CA

Abstract: Amyloid precursor protein (APP) is the precursor to Aβ plaques. The cytoplasmic domain of APP mediates cargo-motor attachments for axonal transport. In APP-KO mice transport is decreased. In old transgenic mice expressing mutated human (APP^{SwInd}) linked to Familial Alzheimer's disease, with both over-expression of mutated protein and plaques, axonal transport is altered, as detected by time-lapse manganese-enhanced magnetic resonance imaging (MEMRI) of the brain in living mice. To answer whether over-expression of expression of mutated APP increases anterograde transport in the absence of plaque we developed a Tet-off system to decouple expression of APP^{SwInd} from plaque, and then studied hippocampal to forebrain transport with MEMRI. APP^{SwInd} expression was suppressed with doxycycline from conception until 10 week of age or not suppressed. Time-lapse MR images were captured before and a successive time points after stereotactic injection of Mn^{2+} (3-5nL) into CA3 of the hippocampus. Images of multiple individuals from the two groups (suppressed or expressed) were aligned and processed with our automated computational pipeline, and voxel-wise statistical parametric mapping (SPM) performed. Brains were harvested after imaging for biochemistry or histopathology. Paired T-tests within-group between time points (0.005 FDR) support the impression that APP^{SwInd} expression alone may affect transport destinations and increase rates of Mn^{2+} accumulation. Histology and biochemistry showed that APP^{SwInd} was expressed 3.2-fold over normal at sacrifice after only 2 weeks release from doxycycline, and no plaques formed. Isolated hippocampal vesicles contained Mn^{2+} and were transported in the squid giant axon at fast transport rates. These surprising results implicate APP^{SwInd} in transport defects, separable from the effect of plaque, and further argue that mouse models over-expressing mutant APP may be partly rescued from the deleterious effect of plaque on transport and cognition.

Disclosures: **E.L. Bearer:** None. **C.S. Medina:** None. **R.E. Jacobs:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.04/C56

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

Support: Tata Innovation Award (Dr. Mandal) Award No.BT/HRD/01/ 05/2015)
(Grant no BT/Indo-Aus/10/31/ 2016/PKM)

Title: Brain hippocampal glutathione level and pH interrelation in Alzheimer's disease: A multinuclear MR spectroscopic cross-sectional study

Authors: D. SHUKLA[1], *P. K. MANDAL[1,2], M. TRIPATHI[3], R. MISHRA[1], A. KALYANI[1], K. SANDAL[1], D. DWIVEDI[1];
[1]Natl. Brain Res. Ctr., Gurgaon, India; [2]Neurodegeneration, FLOREY Inst. of Neurosci. and Mental Hlth., Melbourne, Australia; [3]Neurol., All India Inst. of Med. Sci., Delhi, India

Abstract: INTRODUCTION: Oxidative stress is an important event in Alzheimers Disease (AD). It has been shown during transition from healthy old (HO) to mild cognitive impairment (MCI), the GSH (closed-form) depletes significantly in the hippocampus. Whereas, the significant reduction of GSH level in frontal cortex has been associated with MCI to AD conversion. Likewise, brain pH in the left (LH) and right (RH) hippocampus showed inconsistent changes towards acidic and alkaline range in the aging and AD progression respectively. Therefore, it is important to investigate the effect of brain GSH alteration and pH in the hippocampal areas.
METHODS: We conducted a cross-sectional study with combined ^{1}H MRS and ^{31}P 2D-MRSI on 29 HO, 13 MCI and 24 AD participants, using 3T MR scanner (Achieva, Philips) equipped with a dual tuned (^{1}H/^{31}P) transmit/receive volume head coil (Rapid GmbH, Germany). *In vivo* GSH in LH and RH were estimated among the three study groups using ^{1}H MEGA-PRESS (ON = 4.40 ppm, OFF = 5.00 ppm, TE = 120 ms and TR = 2500 ms, voxel size = 25 × 25 × 25 mm^3) and hippocampal pH using ^{31}P MRSI (TE = 1.39 ms, TR = 1000 ms, FOV = 240 × 240, matrix = 24 × 24, slice thickness = 30 mm). T2-weighted MRI in axial, coronal and sagittal planes were acquired for anatomical reference. Absolute *in vivo* GSH quantitation in mM was performed with external calibration involving T1 and T2 relaxation time corrections.
RESULTS: GSH concentration in study sample having mean-age of 67.41 years was markedly reduced in both LH and RH regions, among MCI and AD as compared to HO ($p < 0.05$), based on a one-way ANOVA, but no significant difference in pH was observed (Figure 1). The association between GSH and pH for both regions was assessed using Pearson correlation coefficient. GSH concentrations in LH and RH were reasonably positive correlated (r = 0.440),

while, pH showed a weak correlation (r = 0.084). The GSH and pH were weakly negative correlated in LH (r = - 0.147) as well as in RH region (r = - 0.265).
CONCLUSION: Depletion of GSH and elevated pH in hippocampus region will contribute to the early diagnosis for the conversion of normal to AD.

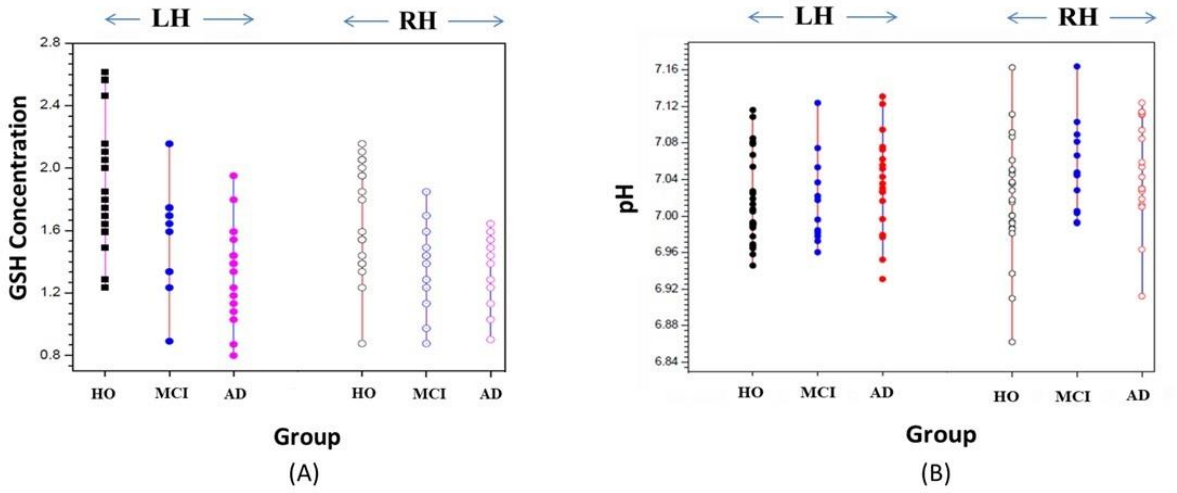

Figure 1: Alteration of (A) GSH concentration level and (B) pH in the left and right hippocampus region among healthy old (HO), mild cognitive impairment (MCI) and Alzheimer's disease (AD) groups.

Disclosures: D. Shukla: None. **P.K. Mandal:** None. **M. Tripathi:** None. **R. Mishra:** None. **A. Kalyani:** None. **K. Sandal:** None. **D. Dwivedi:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.05/C57

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

Support: National Institute on Aging R03 RAG060263A
Canada First Research Excellence Fund

Title: Basal forebrain volume selectively and reliably predicts the cortical spread of Alzheimer's degeneration

Authors: S. FERNÁNDEZ-CABELLO[1], R. N. SPRENG[2], ***T. W. SCHMITZ**[3];
[1]Dept. of Psychology, Univ. of Salzburg, Salzburg, Austria; [2]Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada; [3]Dept. of Physiol. and Pharmacol., Western Univ., London, ON, Canada

Abstract: Alzheimer's disease (AD) neuropathology is thought to spread across anatomically and functionally connected brain regions. However, the precise sequence of spread across different brain regions remains ambiguous. The prevailing model posits that AD pathologies, including neuronal deposition of insoluble amyloid (Aβ) and hyperphosphorylated tau (pTau), starts in the entorhinal cortices, before spreading to interconnected areas of medial temporal and posterior parietal cortex. Challenging this model, we recently provided evidence that degeneration within the nucleus basalis of Meynert (NbM), a subregion of the basal forebrain heavily populated (90% of cell bodies) by cortically projecting cholinergic neurons, precedes and predicts entorhinal degeneration (Schmitz and Spreng, 2016).
There have been few systematic attempts at directly comparing staging models using *in vivo* longitudinal biomarker data, and none to our knowledge which examined if model evidence generalized across independent samples. We therefore used two independent datasets from the Alzheimer's disease Neuroimaging Initiative (*N1* = 284, *N2* = 553), with harmonized cerebrospinal fluid (CSF) assays of Aβ and pTau, and longitudinal structural MRI data over two years. We used voxel-based morphometry to derive longitudinal measures of gray matter degeneration in a priori NbM and the entorhinal regions of interest. To examine the spreading of degeneration, we used a predictive modelling strategy which tests whether baseline gray matter volume in a seed region accounts for longitudinal change in a target region. We demonstrated that predictive pathological spread favored the NbM→entorhinal over the entorhinal→NbM model. This evidence generalized across the independent samples. We also show that CSF concentrations of pTau/Aβ moderated the observed predictive relationship, consistent with an underlying trans-synaptic mechanism of pathophysiological spread. This effect was robust to additional factors, including clinical diagnosis and apolipoprotein genotype. We then applied our predictive modelling strategy to an exploratory whole-brain voxel-wise analysis. The NbM model largely recapitulated its predictive relationship over entorhinal degeneration. The entorhinal model revealed degeneration in areas of the temporoparietal cortices, consistent with the prior entorhinal origin staging model.
Our findings suggest that degeneration of the basal forebrain cholinergic projection system is a robust and reliable upstream event of entorhinal and neocortical degeneration, calling into question the prevailing view of Alzheimer's disease pathogenesis.

Disclosures: S. Fernández-Cabello: None. **R.N. Spreng:** None. **T.W. Schmitz:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.06/C58

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

Support: British Academy Postdoctoral Fellowship (PF160048)

Wellcome Trust (103838)
Cambridge NIHR Biomedical Research Centre

Title: Brain functional network integrity sustains cognitive function despite atrophy in presymptomatic genetic frontotemporal dementia

Authors: *K. A. TSVETANOV, S. GAZZINA, S. JONES, J. B. ROWE;
Cambridge Univ. Dept. Clin. Neurosciences, Cambridge, United Kingdom

Abstract: Frontotemporal dementia (FTD) shows autosomal dominant transmission in up to a third of families, enabling the study of presymptomatic and prodromal phases. Despite self-reported well-being and normal daily cognitive functioning, structural magnetic resonance imaging changes are evident a decade or more before the expected onset of disease. This divergence between cognitive function and brain structure contrasts with the coupling of structure and functional change in symptomatic disease. In healthy physiological ageing, it has been demonstrated that functional specialization rather than structural integrity is the best predictor of cognitive ability. We therefore proposed that in the presymptomatic phase of genetic FTD, the maintenance of brain functional network integrity enables mutation carriers to maintain cognitive performance despite progressive brain atrophy.

We assessed functional connectivity within and between four key large-scale networks (salience network, frontoparietal network, dorsal attention network and default mode network) using task-free functional magnetic resonance imaging in 121 presymptomatic mutation carriers and 134 family members without mutations. Grey matter volumes were quantified from T1-weighted images and cognitive function was assessed using a battery of 13 neuropsychological tests. We examined the differences between mutation carriers and non-carriers on brain structure, functional network organisation and cognition. Because both cognitive profiles and connectivity profiles are multivariate, we used partial least squares models and multiple linear regression models for each modality.

We confirmed group differences in brain structure and function, in the absence of a difference in cognitive performance. However, we also identified behaviourally-relevant structural and functional components. The relationship between structure and cognition was similar in both groups, but the coupling between function and cognition was stronger for carriers than for non-carriers, and increased for carriers approaching the expected onset of disease.

We propose that maintenance of brain functional network connectivity becomes increasingly important for carriers to maintain cognitive performance in the presence of progressive brain atrophy. Although this adaptive response is ultimately overwhelmed, the results have implications for the design of presymptomatic disease-modifying therapy trials and give hope for the ability to maintain function in the presence of FTD neuropathology.

Disclosures: **K.A. Tsvetanov:** None. **S. Gazzina:** None. **S. Jones:** None. **J.B. Rowe:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.07/C59

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

Support: Canadian Consortium for Neurodegeneration and Aging (CCNA)
CIHR
CFI
NSERC

Title: Regional lipid expression abnormalities identified using imaging mass spectrometry correspond to MRI-defined white matter hyperintensities within post-mortem human brain tissue

Authors: *W. PINSKY[1], A. HARRIS[2], A. ROSEBOROUGH[1,3], S. WHITEHEAD[1,3];
[1]Anat. and Cell Biol., [2]Chem., Univ. of Western Ontario, London, ON, Canada; [3]Schulich Sch. of Med. and Dent., London, ON, Canada

Abstract: White matter hyperintensities (WMHs) are a neurological feature of magnetic resonance imaging (MRI) that are clinically associated with an increased risk of stroke and dementia. WMHs represent regions of myelin and axon rarefaction, however, changes in lipid expression underpinning them remain unknown. Alterations in neural lipid expression have been associated with the neuropathology of numerous neurodegenerative diseases, including Alzheimer’s disease and stroke. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) is a potent method for high-throughput analysis of lipid expression and distribution *in situ* that has yet to be applied for lipid analysis of WMHs. For the first time, we successfully demonstrate the application of MALDI IMS for comparative investigation of lipid expression profiles occurring in WMHs and adjacent normal-appearing white matter (NAWM) of post-mortem human brains. MALDI IMS scans conducted in positive or negative ion detection mode on post-mortem human brain tissue sublimated with 2,5-dihydroxybenzoic acid (DHB) or 1,5- diaminonaphthalene (DAN) matrices, respectively, were effective for our untagged, shotgun-style approach to lipid analysis. Using MALDI-IMS analysis, we were able to distinguish regional lipid expression abnormalities corresponding to MRI-defined WMH and NAWM regions. Lipid expression abnormalities include ganglioside species and phospholipid species suggesting altered plasma membrane composition in WMHs. Expanding our understanding of differences in lipid expression will provide greater knowledge of molecular mechanisms underpinning WMHs and provides potential lipid targets for therapeutic intervention.

Disclosures: W. Pinsky: None. **A. Harris:** None. **A. Roseborough:** None. **S. Whitehead:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.08/C60

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

Support: Canada First Research Excellence Fund to BrainsCAN

Title: Blood brain barrier dysfunction and fibrinogen extravasation are associated with post-mortem MR imaging of white matter hyperintensities in normal aging, Alzheimer's disease and cerebrovascular disease

Authors: *A. ROSEBOROUGH[1], K. LANGDON[2], R. HAMMOND[2], S. PASTERNAK[5,3,6], A. KHAN[5,4], S. WHITEHEAD[1,6];
[1]Dept. of Anat. and Cell Biol., [2]Pathology and Lab. Med., [3]Clin. Neurolog. Sci., [4]Med. Biophysics, Schulich Sch. of Med., London, ON, Canada; [5]Robarts Res. Inst., London, ON, Canada; [6]Lawson Hlth. Res. Inst., London, ON, Canada

Abstract: The white matter of the brain is crucial for maintaining cognitive function, and damage to the white matter has been associated with aging, cognitive decline and the progression of dementia. Clinically, white matter damage is detected using Magnetic Resonance Imaging (MRI), where abnormal regions appear as white matter hyperintensities (WMH). WMH result from chronic ischemia and hypoperfusion of the white matter due to cerebral small vessel disease (SVD). Despite imaging and pathological studies, the mechanisms linking SVD and rarefaction of the white matter remain poorly understood. It has been proposed that microvascular stenosis and disruptions of the blood brain barrier may play a role in the loss of myelin and axonal density. The goal of this study was to investigate associations between vascular disease within WMH, evidence of blood brain barrier (BBB) dysregulation and reductions in white matter density. Twenty post-mortem brains with a neuropathological diagnosis of normal, Alzheimer's disease (AD) and/or cerebrovascular disease (CVD) were imaged using 7 Tesla MRI. T1, T2 and FLAIR images were acquired for the identification of WMH. Scans were rated based on the severity of WMH and on the presence of periventricular infarction identified as fluid-filled cavities within the WMH. Tissue blocks corresponding to areas of WMH and normal appearing white matter were collected for further histological and immunohistochemical (IHC) analysis. The stenotic indices (SI) of small, medium and large arterioles and venules were calculated within WMH and normal-appearing white matter. SI of the small venules and arterioles was associated with axonal and myelin loss as well as periventricular infarction. To investigate BBB dysfunction, IHC for the serum protein fibrinogen revealed extravasation from venule and arterioles and uptake into glial cells, which was confirmed using dual-labelling IHC with Olig2 and GFAP to determine cell lineage. The degree of intra-glial fibrinogen accumulation within the

white matter was significantly associated with microvascular stenosis, white matter rarefaction and the presence of periventricular infarction. These results suggest that stenosis of both the arterioles and venules is associated with fibrinogen accumulation in the subcortical white matter extending beyond the borders of MRI-visible lesions. WMH often accumulate in mid-life and precede cognitive decline, therefore an understanding of the mechanisms by which SVD relates to white matter toxicity, potentially through fibrinogen leakage, represent an important area of future study.

Disclosures: **A. Roseborough:** None. **K. Langdon:** None. **R. Hammond:** None. **S. Pasternak:** None. **A. Khan:** None. **S. Whitehead:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.09/C61

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

Title: Pathological mechanism and therapy for extracellular space in Alzheimer's disease

Authors: *D. CHUI[1], X. YUE[2], Z. TONG[2], A. WANG[1], R. WANG[1], Y. JIN[1], H. HAN[1]; [1]Beijing Key Lab. of Magnetic Resonance Imaging, Peking Univ. Third Hosp., Beijing City, China; [2]Lab. of Alzheimer’s Optoelectric Therapy, Alzheimer’s disease Ctr., Capital Med. Univ., Beijing City, China

Abstract: With the aging of the population, more and more elderly people will face diseases, including Alzheimer's disease (AD) and Vascular-associated dementia (VaD). The most common age-related neurodegenerative disease is AD, the imbalance between Aβ generation and clearance accumulated in extracellular plaques. The sporadic form of AD is characterized by an overall impairment in Aβ clearance. Strategies that promote local growth of lymphatic vessels have the potential to improve clearance Aβ by meningeal lymphatics. whether enhancing clearance at the blood-brain barrier can improve lymphatic drainage function, remains to be addressed. Understanding substance transportation in brain extracellular space (ECS), especially in deep brain is essential for the complete answer to the question of how brain functions in the absence of a lymphatic drainage pathway. The brain interstitial fluid (ISF) drainage in deep brain was recently studied using a tracer-based MRI method, and a non-uniform ISF drainage was demonstrated with various distribution territories and movement speeds in different regions. The regulating and the barrier effects of myelin were unchanged under different local interstitial pressures of the ECS and in AQP4-knockout rats, but were impaired as the integrity of boundary structure of drainage system was destroyed in a demyelinated rat model. We thus proposed that the brain homeostasis be maintained within each ISF drainage division locally, rather than across the brain as a whole. In addition, Aβ deposition in the ECS and rescued memory in an APP/PS1

transgenic mouse of AD model, our findings are expected to have a potentially significant influence on brain-inspired and artificial intelligence, the future of which is very promising. Immunotherapy and local brain drug delivery via the brain ECS could circumvent the BBB and reduce systemic toxicity. Updated knowledge of the whole-brain ISF drainage system (ISS) provides a beneficial reference for improving the immunotherapeutic efficacy via ISS. New insights into how behave our and genetics modify ECS-ISS function should lead to the development of new preventive tools for PCAD and novel Immunotherapy targeting Aβ clearance therapeutic targets.
Acknowledgments: This work was supported by the NBRD Program of China (2016YFC1306302, 2016YFC1305903) and the NNSF of China (81571044, 81471633, 61450004 and 81171015).

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Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.10/C62

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

Title: Modulation of ventral visual pathways connectivity: Cortical interactions evaluated for structural alterations in visual perceptual abnormalities in Parkinson’s disease

Authors: *G. ELUMALAI[1], P. MAITI[2], G. VINODHANAND[1], C. VADIYALA[1], D. SINGH[1], N. DAYAL[1], V. L. BROWN[1], V. KURRA[1];
[1]Anantomy and Neurosci., Team NeurON, Col. of Medicine, Texila American Univ., Georgetown, Guyana; [2]Neurosci., Brain Res. Laboratory, Saginaw Valley State Univ., Saginaw, MI

Abstract: INTRODUCTION: Patients with Parkinson’s disease have a number of specific visual disturbances. These include changes in color vision, contrast sensitivity, and difficulty in perceiving the orientation of lines, edges and object perception [1]. All these visual processing’s are controlled by the ventral stream which identifies features of objects, passes from V1 through areas V2 and V4 to the inferior temporal cortex [1]. The study here is focused on correlating the visual functional deficits with neural structural connectivity in Parkinson’s patients using "Diffusion Tensor Imaging Tractography". The study involves 120 DTI datasets, both the sexes of Control and disease progression stages of PD, with age ranges from (60 to 120) years.
RESULTS: On observation, the females displayed with increase number of fibers in control when compared with male, but not much difference was seen in right and left hemisphere in both sexes. Males show progressive decrease in the (number and volume) of fibers in right,

hemispheric among the cognitive declining stages of PD. The results are statistically significant at $P<0.05$.

CONCLUSION: On current observations, it was noticed deterioration in the ventral stream pathway were identified, and is supposed as an underlying cause for visual disturbances like color vision and object perception in Parkinson's patients. It is also observed that males show progressive decrease in the (number and volume) fibers among the cognitive declining stages of PD. However, for better understanding of this findings, functional and effective connectivity analysis must be recommended.

References:

[1] Weil, R. S., Schrag, A. E., et al. (2016) Visual dysfunction in Parkinson's disease. Brain, Volume 139, Issue 11, November 2016, Pages 2827-2843.

[2] Golomb, A. C., (2010) Parkinson's Disease as a Disconnection Syndrome. Neuropsychol Rev. Jun; 20(2): 191-208.

[3] Greene, J.D.W., Dr. John, D.W.G.,(2005) Apraxia, agnosias, and higher visual function abnormalities. J Neurol Neurosurg Psychiatry ;2005:76

[4] Heutink,j., Indorf. D.L., & Cordes,C,.(2017) The neuropsychological rehabilitation of visual agnosia and Balint's syndrome. Neuropsychological Rehabilitation

Disclosures: G. Elumalai: None. **P. Maiti:** None. **G. Vinodhanand:** None. **C. Vadiyala:** None. **D. Singh:** None. **N. Dayal:** None. **V.L. Brown:** None. **V. Kurra:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.11/C63

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

Title: Optic ataxia in Alzheimer's: Neural-cortical connectivity analysis in correlations with "how" stream visual pathways in disease progression stages of Alzheimer

Authors: *N. H. C. CERESOLI[1], **N. DYAL**[2], G. ELUMALAI[3], V. KURRA[4], G. VINODHANAND[5], C. VADIYALA[6];

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Abstract: Optic ataxia is a neurological condition, manifestations with disturbances in visual guided hand movements on reaching a target object. It is more prevalent in Alzheimer Patients, and previous studies has failed to provide a substantial evidences for neural structural relations to this symptoms.

Methods: In this research, Team NeurON, attempted to correlate the dorsal stream visual pathway with Optic Ataxia in Alzheimer's Patients. The study was carried out through "Diffusion Imaging Fiber Tractography" which involves 60 DTI datasets from control and Alzheimer Patients (50-70 yrs) with the symptoms of Optic Ataxia. The fibers were traced, and confirmed the structural alterations and their underlying substrates for Optic Ataxia, in correlations with "How" stream Visual Pathways from Visual cortex (BA 17,18 &19) to Superior Parietal Lobule (BA 7).
Observations: It was observed that fibers of the females of the control group was higher when compared to the males. However, on completion, it was noted that females displayed more plummet changes in numbers and volumes of "how stream - visuomotor coordination pathway", when compared to the males.
Results: In conclusion, based on our observations, destructions in the visuomotor coordination pathway were identified, and is believed as an underlying substrates for Optic ataxia in Alzheimer patients. However, for better understanding of this findings, functional and effective connectivity analysis must be recommended.

Disclosures: N.H.C. Ceresoli: None. **N. Dyal:** None. **G. Elumalai:** None. **V. Kurra:** None. **G. Vinodhanand:** None. **C. Vadiyala:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.12/C64

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

Title: Visual processing of spatial recognition: Cortical connectivity deficits in Parkinson’s spatial perceptual visual pathway

Authors: G. VINODHANAND[1], G. ELUMALAI[1], **P. MAITI**[2], D. SINGH[1], N. DAYAL[1], V. BROWN[1], *Z. L. GODLO[1], C. VADIYALA[1], V. KURRA[1];
[1]Texila American Univ., Georgetown, Guyana; [2]Saginaw Valley State Univ., Saginaw, MI

Abstract: Introduction: Parkinson’s disease (PD) is a Neurodegenerative disorder traditionally linked solely to motor impairment but recent findings have revealed that patients suffering from PD exhibit decline in visual-spatial skills [1]. Previous studies have explored fronto-striatal connectivity grey matter atrophy of temporo-parietal cortical regions might account for these deficits in visual-spatial skills but those results were inconsistent and inconclusive [2]. The aim of this study is to explore the neural structural connectivity between the superior parietal lobe (BA 5) and the primary visual cortex (BA 17, 18 & 19) in PD patients. Generally, this pathway modulates visual and spatial domains, hence if found to be impaired in PD patients could explain the dysfunction in visual-spatial skills.

Methods: In this research, Team NeurON, attempted to correlate the dorsal stream visual pathway in Parkinson's Patients. The study was carried out through "Diffusion Imaging Fiber Tractography" which involves 120 DTI data sets from control and Parkinson's Patients between 60-120 years. The fibers were traced, and confirmed the structural alterations in the Visual Pathways from Visual cortex (BA 17,18 &19) to Superior Parietal Lobe (BA 5).
Observations: It was observed that fibers in the females of the control group were higher when compared to that of the males, and on completion, males displayed more prominent changes in numbers and volumes of where stream pathway". In males both right and left hemisphere were affected, where as with female much difference is not noticed . The results are statistically significant at $P<0.05$.
Results: In conclusion, based on our observations, destruction in the where stream pathway were identified, and is believed as an underlying cause for deficiency in visual spatial recognition. However, for better understanding of this findings, functional and effective connectivity analysis must be recommended.
References:
[1]. Crucian, G. P. & Okun, M. S. (2003). Visual-Spatial Ability In Parkinson's Disease. Frontiers in Bioscience, 8: 992-997.
[2]. Ekker, M. S., Janssen, S., et al. (2010). Ocular and visual disorders in Parkinson's Disease: Common but frequently overlooked. Parkinson and related disorders, 40: 1-10.
[3]. Caproni,S., Macro,M., et al. (2014) Subclinical Visuospatial Impairment in Parkinson's Disease: The Role of Basal Ganglia and Limbic System Frontiers of science. 5: 152.
[4]. Possin,K,L.,(2010) Visual Spatial Cognition in Neurodegenerative Disease Neurocase. Dec; 16(6): 466-487.

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Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.13/C65

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

Title: Associative visual object agnosia (AVOA): Structural alterations and their underlying substrates in correlations with what stream visual pathways in Alzheimer's

Authors: D. SINGH[1], G. ELUMALAI[1], P. MAITI[2], G. VINODHANAND[1], N. DYAL[1], V. L. C. BROWN[1], N. A. S. V. GHANTA[1], *M. NANDURI[1];
[1]Texila American Univ., Georgetown, Guyana; [2]Saginaw Valley State Univ., Saginaw, MI

Abstract: INTRODUCTION: Visual agnosia is a promptly noticed neurological deficit in Alzheimer's disease (AD), affects the inferior temporal lobe, which leads an inability to perceive objects. The interconnection between the two cortices in the visual ventral stream, the visual cortex (BA 17, 18, 19) and inferior temporal lobe (BA 20) explain how we perceive and recognize the objects, called associative object visual perception. We Team NeurON focused on correlating the neural structural connectivity with associative visual agnosia in Alzheimer's patients using "Diffusion Tensor Imaging Tractography". The study involves fifty DTI datasets, both the sexes of Control and disease progression stages of AD, with age ranges from 50 to 90 years.
RESULTS: On observation, the females displayed with a progressive increase in the (number and volume) fibers among the cognitive declining stages of AD. But, the males, noticed with a bi-modal variation (number and volume) of fibers, within the disease progression stages of AD.
CONCLUSION: Although the statistical analysis was insignificant, it was noticed that the males are predominantly affected than females. The current observations, propose an insight to understand the bi-modal distribution of fibers in the male and progressive increase of fibers in the female, from the control to disease progression stages of AD. However, these findings need to be confirmed with functional and effective connectivity analysis for further understandings.

Disclosures: D. Singh: None. **G. Elumalai:** None. **P. Maiti:** None. **G. Vinodhanand:** None. **N. Dyal:** None. **V.L.C. Brown:** None. **N.A.S.V. Ghanta:** None. **M. Nanduri:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.14/C66

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

Title: Diffusion imaging fiber tractography: Prosopagnosia and facial expression analysis deficit in progressive Alzheimer’s

Authors: *C. VADIYALA[1], P. MAITI[3], G. ELUMALAI[2], P. SINGRU[1], A. O. HAUGHTON[4]; [1]Dept. of Anatomy, Col. of Med., Texila American Univ., Georgetown, Guyana; [2]Anat. and Neurosci., Texila American Univ., Kakinada (Urban), India; [3]Psychology and Neurosci. Program, Central Michigan University/St. Mary's of Michigan, Saginaw, MI; [4]Dept. of Anatomy, Col. of Med., Texila Ameriican Univ., Georgetown, Guyana

Abstract: INTRODUCTION: Prosopagnosia is a common sign in Alzheimer’s Disease (AD), characterized by visual deficits with loss of familiar face recognition and facial expression processing. Invariant aspects of the face are analyzed by Fusiform Face Area (FFA), located in the lateral fusiform gyrus and variant aspects like eye gaze, expression, lip movements facilitating social communication by Superior Temporal Sulcus (STS). In Alzheimer’s, atrophy

of FFA and STS are understood to cause prosopagnosia and failure of facial expression analysis. We used 72 Diffusion Tensor Imaging (DTI) datasets (36 Males and 36 Females), with the age range (60-120) years. This Study aimed in identification and analysis of neural structural connectivity responsible for prosopagnosia and facial expression analysis. Also, correlates functional importance, by *"Non-Invasive Diffusion Imaging fiber Tractography".*
RESULTS: Tractography reveals highest number of fibers in the tracts of females than males. The tract from visual cortex to the FFA show increased number of fibers, in AD and in progressing stages, than control in males and females respectively. Tract from visual cortex to the STS revealed highest number of fibers in progressing stages and rigid detoriation is observed at AD in males and females. On Bi-hemispherical analysis, affect of AD is observed on left hemisphere for females and right for males. Results are Statistically significant for the both tracts at $p<0.001$.
CONCLUSION: Prosopagnosia in Alzheimer's is due to the decreased nerve fibers to the FFA as believed but Failure of facial expression is not only concerned with cognitive function, cortical tract detoriation is also observed.
REFERENCES:
1.Michael. E., Steven. Y. (16 September 2009); Perceptual Expectation Evokes Category-Selective Cortical Activity. *Cerebral Cortex*, 20 (5),1245-1253
2.Justine. s., Shinsuke. o., Brennan. m. (01 February 1992); Functional Neuroanatomy of face and object processing: a positron emission tomography study. Brain,115(1), 15-36
3.John R. Hodges., Nelson Butters., David P. Salmon.(August 1993); Recognition and naming of famous faces in Alzheimer's disease: A cognitive analysis. Neuropsycholgia, 31(8),775-788
4.J. J. Evans., A. J. Heggs., N. Antoun., J.R. Hodges. (01 February 1995); Progressive prosopagnosia associated with selective right temporal lobe atrophy: A new syndrome? *Brain*,118(1),1-13

Disclosures: C. Vadiyala: None. **P. Maiti:** None. **G. Elumalai:** None. **P. Singru:** None. **A.O. Haughton:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.15/C67

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

Title: Logopenic aphasia tau pathology: An observation on phonological loop fiber-specific white matter reductions in Alzheimer's disease - Is it a causal or casual link

Authors: *V. KURRA[1], G. ELUMALAI[2], P. MAITI[5], G. VINODHANAND[3], C. VADIYALA[4];

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Abstract: INTRODUCTION: Logopenic Aphasia (LA), a variant of primary progressive aphasia characterized with difficulty in retrieving precise words, names, or numbers and sentence repetition. Previous studies detailed that 50% of LA patients had (Alzheimer's disease) AD pathology, and characteristics of LA synchronize with language impairments in AD. Researchers believed that, the atrophy of Phonological loop (A sensorimotor circuit that includes auditory regions, the inferior parietal lobe, and Broca's area, which integrates phonological processing and executes motor output) cause LA. Methodology: We focused on structural connectivity of Phonological loop using "Diffusion Imaging fiber Tractography" with 60 DTI data sets (30 Males and 30 Females) of both control and progressive stages of Alzheimer's, with the age range 55-120 years, and made an attempt to correlate the Logopenic aphasia, with phonological loop fiber-specific white matter reductions in early AD.
RESULTS: Overall progressive diminution were observed in the phonological loop of males and left hemispheric deterioration is markedly seen in terms of both fibers and tract volume (significant at $p < 0.05$). Current study, also reveals that contralateral adaptation are more pronounced in AD males than in females AD.
CONCLUSION: Based on our analysis on phonological loop in AD, Logopenic aphasia may present as clinical marker for early Alzheimer's. These findings must be vindicated with functional MRI analysis.
KEYWORDS: Logopenic Aphasia, Diffusion Imaging fiber Tractography, Wernicke's area, Broca's area, phonological loop tract.

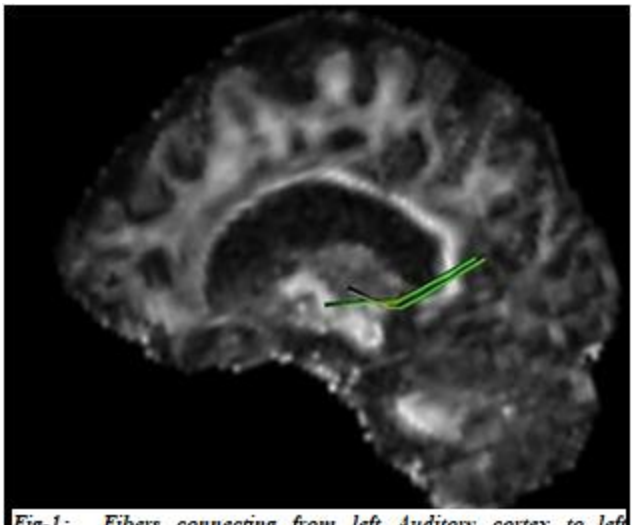

Fig-1: Fibers connecting from left Auditory cortex to left wernicke's area in AD stage of male alzheimer's patients in saggitalview

Disclosures: V. Kurra: None. **G. Elumalai:** None. **P. Maiti:** None. **G. Vinodhanand:** None. **C. Vadiyala:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.16/C68

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

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Title: Neural activity patterns underlying abnormal phonological processing in patients with logopenic variant primary progressive aphasia

Authors: *K. RANASINGHE[1], A. J. BEAGLE[2], D. MIZUIRI[2], S. HONMA[2], A. WELCH[2], M. GORNO-TEMPINI[2], K. A. VOSSEL[5], J. F. HOUDE[3], S. S. NAGARAJAN[4];
[1]Univ. of California San Francisco, San Francisco, CA; [3]Dept of Otolaryngology -- Head and Neck Surgery, [4]Radiology and Biomed. Imaging, [2]UCSF, San Francisco, CA; [5]Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Logopenic variant (lvPPA) is one of the subtypes of primary progressive aphasia (PPA) syndrome of which the most frequent underlying pathology is Alzheimer's disease. The patients with lvPPA presents with predominant impairments in the language domain as opposed to other cognitive domains. Specifically, the language impairment in lvPPA is characterized by errors in phonological working memory—a function mapped onto the 'dorsal stream' of language processing network, also known as the phonological loop. Despite compelling evidence from structural and functional neuroimaging studies in patients with lvPPA implicating the early and selective involvement of language network, direct evidence of abnormal phonological loop activity remains elusive. **Methods**: We utilized the millisecond time resolution of magnetoencephalographic imaging (MEGI) to examine the neural activity patterns during auditory stimulus encoding in patients with lvPPA. We hypothesized that auditory stimulus encoding in lvPPA patients would show aberrant spectral patterns localized within the language network, in the oscillatory processes that are typically associated with speech motor control such as beta-power decreases and theta-alpha increases. We compared lvPPA (n=14) patients with age-matched healthy participants (n=13) and also with patients with non-fluent aphasia (nfvPPA; n=14)—another variant of PPA with predominant motor speech errors. Subjects listened to an auditory stimulus consisting of 2-syllables and repeated it, while

lying supine in the scanner. Stimuli were prerecorded and consisted of permutations of /ba/, /da/, and /pa/. We examined source localized event related brain activity in the theta-alpha (4-13 Hz) and beta (13-30 Hz) frequencies during the first 300ms of auditory stimulus encoding. **Results**: Both patient groups showed significant behavioral impairment in syllable repetition (46% and 53% in lvPPA and nfvPPA respectively; 94% in healthy controls; P<0.0001). When compared to age-matched healthy controls, lvPPA patients showed pronounced reduction in theta-alpha frequency within the superior temporal, posterior parietal and dorsal frontal regions. In contrast, nfvPPA patients showed pronounced enhancement of both theta-alpha and beta frequency activity within the anterior temporal/inferior frontal cortex. **Conclusion**: These findings shed new light on potential sources of speech dysfunction in aphasia and neuropsychiatric disorders, identifying anatomically and behaviorally dissociable activation time windows critical for successful speech reproduction.

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Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.17/C69

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

Title: Diffusion imaging fibre tractographic analysis for auditory saccadic attention deficit (ASAD) in progression stages of Alzheimer’s disease

Authors: *P. P. SINGRU[1], G. ELUMALAI[2], C. VADIYALA[3], H. KURRA[1];
[1]Texila American Univ., Georgetown, Guyana; [2]Anat. and Neurosci., Texila American Univ., Kakinada (Urban), India; [3]Texila American Univ., Hyderabad, Telangana, India

Abstract: INTRODUCTION: The ability of human beings to perceive auditory stimulus in everyday acoustic environments depends on the localization and the identification of relevant sounds. Localization (or) the spatial orientation of the stimulus and relative motor output with saccadic eye and neck movements is analyzed by the tract from Auditory cortex (BA-41, 42) to Premotor Eye-Ear Field [PEEF] (BA-8b), called Auditory-saccadic pathway. Our study aimed on comparative analysis of this pathway in control adults with Alzheimer’s Patients, using “Diffusion Imaging fibre Tractography”. We used 60 Diffusion Tensor Imaging (DTI) datasets (30 Males and 30 Females), between the age ranges from 55-100 yrs.
RESULTS: Study reveals that, bihemispheric detoriation of auditory saccadic pathway fibers were observed in females with AD, as compared to males. Whereas the auditory saccadic fibers are markedly increases in disease progression stages of males AD.

CONCLUSION: Present study proves the existence of neurostructural connectivity of Auditory-saccadic pathway (dorsal stream-auditory pathway) and the detoriation of this track leads to cause of Auditory saccadic attention deficit (ASAD) in Alzheimer's Patients. But, the results must to strongly evidence with functional and efficient connectivity analysis in future.
Keywords: Auditory-saccadic pathway, Auditory Attention Deficit, dorsal stream-auditory pathway, Premotor Eye-Ear Field, Auditory cortex.

Disclosures: P.P. Singru: None. **G. Elumalai:** None. **C. Vadiyala:** None. **H. Kurra:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.18/C70

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

Title: Analysis of neural structural connectivity in olfactory attention deficit in Alzheimer's patients as a diagnostic and progression monitoring method

Authors: *H. CHATTERJEE[1], G. VINODHANAND[2], G. ELUMALAI[3], N. S. OSAKWE[4], N. SEWRAM[4];
[1]Texila American Univ., Georgetown, Guyana; [2]Texila American Univ., East Bank Demerera, Guyana; [3]Anat. and Neurosci., Texila American Univ., Kakinada (Urban), India; [4]Texila American Univ., East Bank Demerara, Guyana

Abstract: Olfactory deficits are mostly seen in early mild cognitively impaired (EMCI) patients and in early Alzheimer's disease (AD), like in most neurodegenerative disorders. It is a clinical marker appearing years before declining motor and cognitive functions in AD patients. A study comparing the association of olfactory stimuli with visual response between a control group and AD-affected group, stated that degeneration in the central olfactory areas is the cause of olfactory deficits, but no imaging studies had been performed to note this degeneration or to mark its extent. Identification of this structure could serve as a potential clinical marker for AD severity and help monitor disease progression. This study obtained data from the Alzheimer's disease Neuroimaging Initiative database and uses Diffusion tensor images (DTI) datasets of 72 control and 72 AD affected patients from both the sexes, with ages ranging from 55 to 120 years. The aim of this study was to identify the structure, variation in which gives rise to Olfactory Attention Deficit by identifying the structural connectivity between the Olfactory Cortex and Frontal Eye Field, using DTI fibre tractography to establish an Olfactory-Saccadic pathway. It also performs a comparative analysis of the tracts in progressing stages of AD and focuses hemispheric dominance in all stages. Progressive changes were seen in all stages from control group to advancing stages in both sexes, also a bimodal distribution of fibres was seen in both sexes, but this distribution was more remarkable in the females than in males (Figure 1-3). The

mostly affected hemisphere in all the experimental stages was seen to be the right hemisphere. Therefore, any variations to the structural connections on the right of the brain responsible for conducting olfactory stimuli to produce a saccadic response, may cause olfactory attention deficit in varying extents.

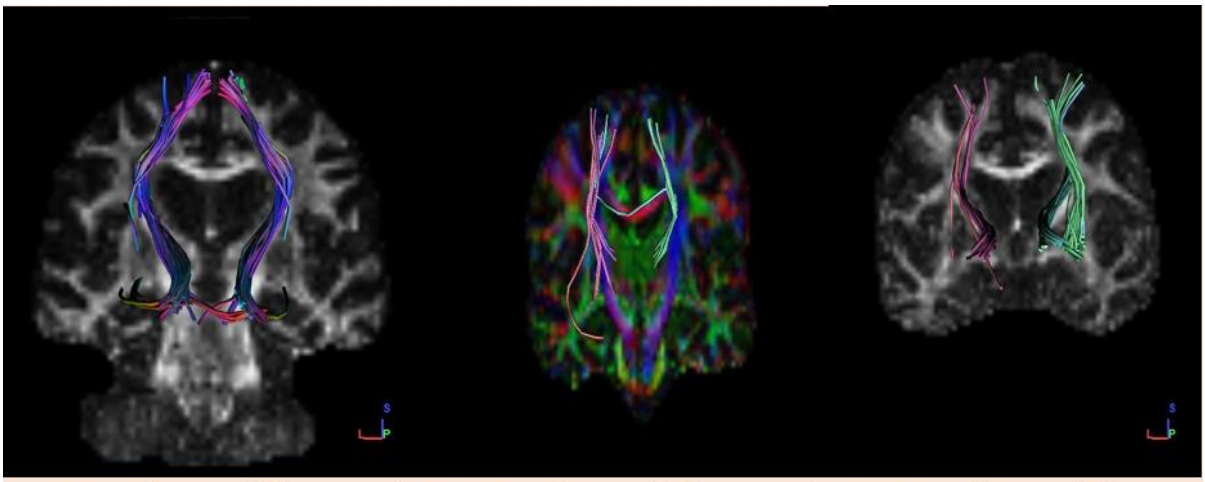


Fig.1 : Coronal section of olfactory visual saccadic pathway in Control – MALE

Fig.2 : Coronal section of olfactory visual saccadic pathway in Early Mild Cognitive Impairment

Fig. 3 : Coronal section of olfactory visual saccadic pathway in Mild Cognitive Impairment

Disclosures: H. Chatterjee: None. **G. Vinodhanand:** None. **G. Elumalai:** None. **N.S. Osakwe:** None. **N. Sewram:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.19/C71

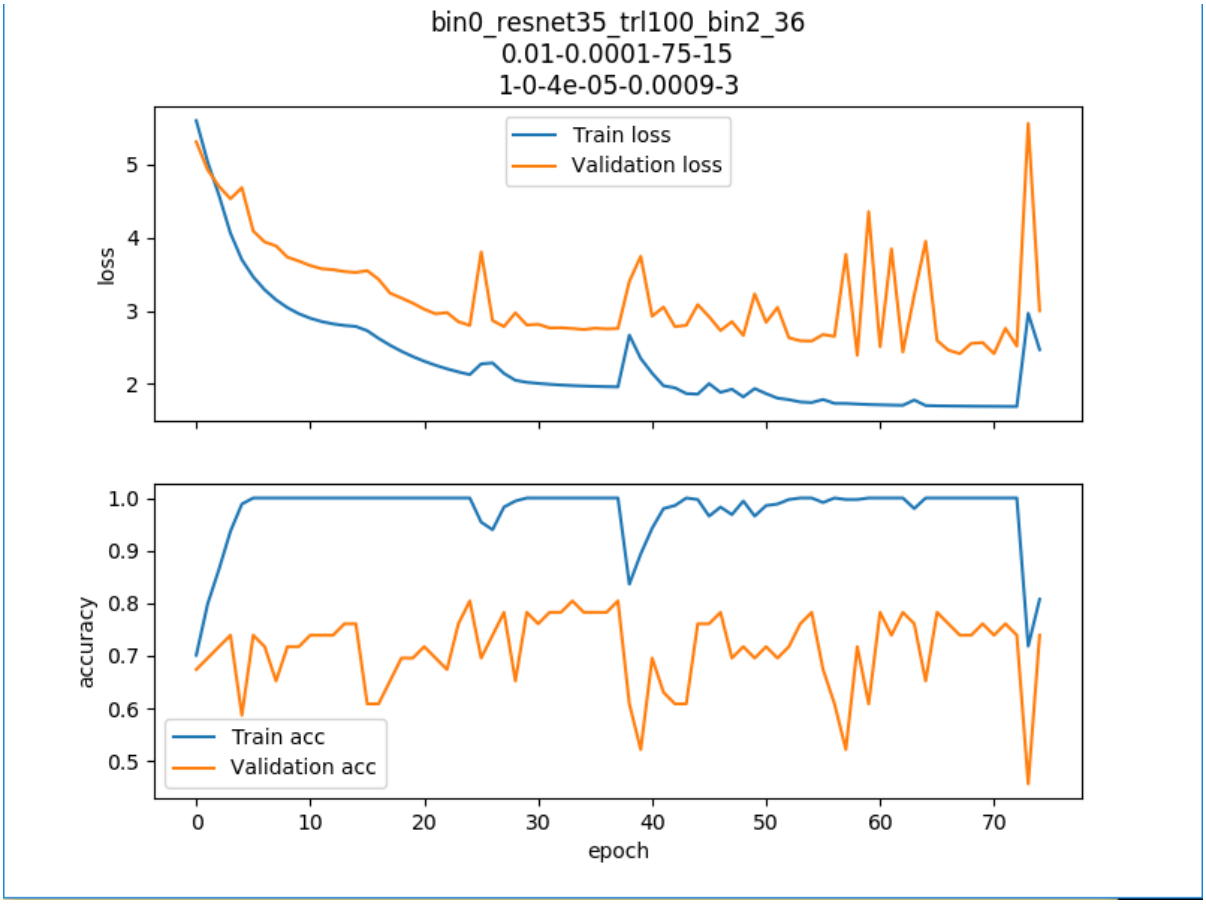
Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Convolutional neural network for predicting conversion from mild cognitive impairment to Alzheimer's dementia using transfer learning

Authors: *J. B. BAE[1], J. STOCKS[2], A. HEYWOOD[2], Y. JUNG[3], P. KARTEEK[4], F. BEG[4], L. WANG[5];
[1]Northwestern Univ., Evanston, IL; [2]Northwestern Univ., Chicago, IL; [3]KAIST, Daejeon, Korea, Republic of; [4]Simon Fraser Univ., Burnaby, BC, Canada; [5]Psychiatry, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Background: Predicting conversion to Dementia of the Alzheimer's Type (DAT) among Mild Cognitive Impairment (MCI) patients is invaluable for patient care, as well as in selection for clinical trials. In order to classify subjects as either stable (sMCI: MCI→MCI) or

progressive (pMCI: MCI→DAT) in which temporal distance of conversion is at 36 months, we developed a 3D-Convolutional Neural Network (3D-CNN) utilizing transfer learning. A classification task of stable Normal Control (sNC) vs. stable Dementia of Alzheimer's Type (sDAT) was firstly conducted with 3D-CNN and this domain knowledge was used as the transfer learning information in the sMCI. vs. pMCI classification task. **Method**: 416 sNC, 311 sDAT, 220 sMCI and 220 pMCI patients' skull-stripped baseline structural MRI (sMRI) available were collected from ADNI and pre-processed by using crop, pad, bias field correction, and affine linear alignment with FMRIB Software Library (FSL). The source classification task, i.e., sNC vs. sDAT, was performed by using ResNet35. The model resulted in 93.58% test set classification accuracy and this obtained domain knowledge, i.e., the model and weight, was transferred to conduct the target task of classifying sMCI vs. pMCI. **Result**: The domain transferred ResNet35 model classifying sMCI vs. pMCI produced test set classification accuracy at 78.26%. This is the highest generalization performance seen in the current literature. **Conclusion**: 3D-CNN ImageNet models with transfer learning successfully classified sMCI vs. pMCI. The use of ResNet architecture and the reasonable amount of test samples ensured the generalizability of performance. These models are easily reproducible. Moreover, unlike previous machine learning methods, it holds the possibility of extracting neuroanatomic features that contribute to AD development.



Disclosures: **J.B. Bae:** None. **J. Stocks:** None. **A. Heywood:** None. **Y. Jung:** None. **P. Karteek:** None. **F. Beg:** None. **L. Wang:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.20/C72

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

Title: An electrochemical sensor array for detecting Alzheimer's disease biomarker VOCs

Authors: *S. EMAM[1], P. P. KULKARNI[4], M. NASROLLAHPOUR[1], B. COLARUSSO[2], A. EKENSEAIR[3], C. F. FERRIS[5], N. SUN[1];
[1]Electrical Engin., [3]Chem. Engin., [2]Northeastern Univ., Boston, MA; [4]Psychology, Northeastern Univ. Dept. of Psychology, Boston, MA; [5]Dept Psychology, Northeastern University, Ctr. for Translational NeuroImaging, Boston, MA

Abstract: Currently more than 16 million Americans provide unpaid care for people with Alzheimer’s disease (AD) or other dementias. For those who pay for assisted living, $341,840 is the estimated average lifetime cost of care for an individual living with dementia. Pathophysiological process of AD typically begins at least a decade before symptoms appear. Early and accurate diagnosis of AD could save 15%, which is about $7.9 trillion in medical, and care costs. We are reporting a fast, low-cost, completely non-invasive and quantitative method for early diagnose of AD through sensing three biomarker volatile organic compounds (VOCs) in the exhaled breath by using novel ultra-sensitive and highly selective electrochemical gas sensors. Our sensors have the size of 155 mm^3 each. These silicon-based sensors have the potential to be used as an electronic device, which means that they can be connected to a micro controller unit to monitor the resistance change and transmit the data to a smartphone through Bluetooth. The breath sensors have shown sensitivity of 20~100 part per trillion (ppt), highly selectivity (one molecule only), and have shown excellent preliminary results on transgenic human APOE knock-in rats as compared to wild-type controls. These sensors with the application of recognizing target molecules includes a layer of molecularly imprinted polymer disposed on a thin layer of graphene -Prussian blue on a silicon substrate. The sensors were tested on 12 rats including, 4 APOE males, 4 APOEfemales and 4 age-matched control male rats. The sensors were placed very close to the rat mouth while it was restrained. All the male APOE4 tested positive. Multimodal MRI showed these males had significant changes in brain morphology and functional coupling together with deficits in cognitive behavior as compared the other experimental groups. All brains are being analyzed for beta amyloid levels and phosphorylated tau. The results indicated that the proposed sensors were sensing the VOCs in the breath of AD rats while remaining unchanged at the time of exposure to the breath of non-AD rats.

Disclosures: **S. Emam:** None. **P.P. Kulkarni:** None. **M. Nasrollahpour:** None. **B. Colarusso:** None. **A. Ekenseair:** None. **C.F. Ferris:** None. **N. Sun:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.21/C73

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

Support: NIH-NIA R01 AG057962
Taub Institute Imaging Core pilot grant.

Title: Validation of a fully automated method for precise quantification of amyloid beta using F18-Florbetaben PET scans

Authors: *M. TAHMI, W. BOUZEID, Q. R. RAZLIGHI;
Neurol., Columbia Univ., New York, NY

Abstract: Background: Precise quantification of Amyloid-β in the brain is of high value as there is an increased interest in the detection of this protein earlier in life as well as understanding the evolution of its accumulation in the brain. In this work, we evaluated and validated an automated method that quantifies Amyloid-B using ^{18}F-Florbetaben PET scans. **Methods:** FreeSurfer neuroanatomical labels were transformed into PET image space. These labels are then used to quantify the accumulation of the Amyloid-β plaques in the brain by dividing Amyloid-β tracer uptake in each voxel of interest by that in a reference region (the Standardized Uptake Value Ratio, or SUVR). We have also evaluated our technique using post-mortem histopathological assessment data from 52 older participants (mean age 79, 6±9.7) who previously had completed both structural MRI and ^{18}F-Florbetaben PET scans. Regional SUVRs were computed in seven regions of interest (ROIs) in which histopathological examination was also performed. Two-way ANOVA and post-hoc slope difference tests were used to assess whether our technique improved the relationship between the PET image quantification results and the histopathological assessment relative to current standard techniques for Amyloid-β PET scan processing. **Results:** Our method resulted in consistently and significantly higher SUVRs in comparison to the conventional method in almost all ROIs ($p<0.010$). In addition, a two-way ANOVA revealed a significant main effect of method ($p<0.0001$) as well as significant effect of method on the relationship between quantified SUVR and histopathological assessment data ($p<0.010$). Post-hoc slope difference tests showed that our technique significantly outperformed the existing conventional method. **Conclusions:** These findings suggest that processing the amyloid PET data in subjects' native space can improve the accuracy of the resulting SUVRs highliting a higher ROI precision. This degree of precision is necessary to provide valid estimates of Amyloid-β that could reflect true disease risk.

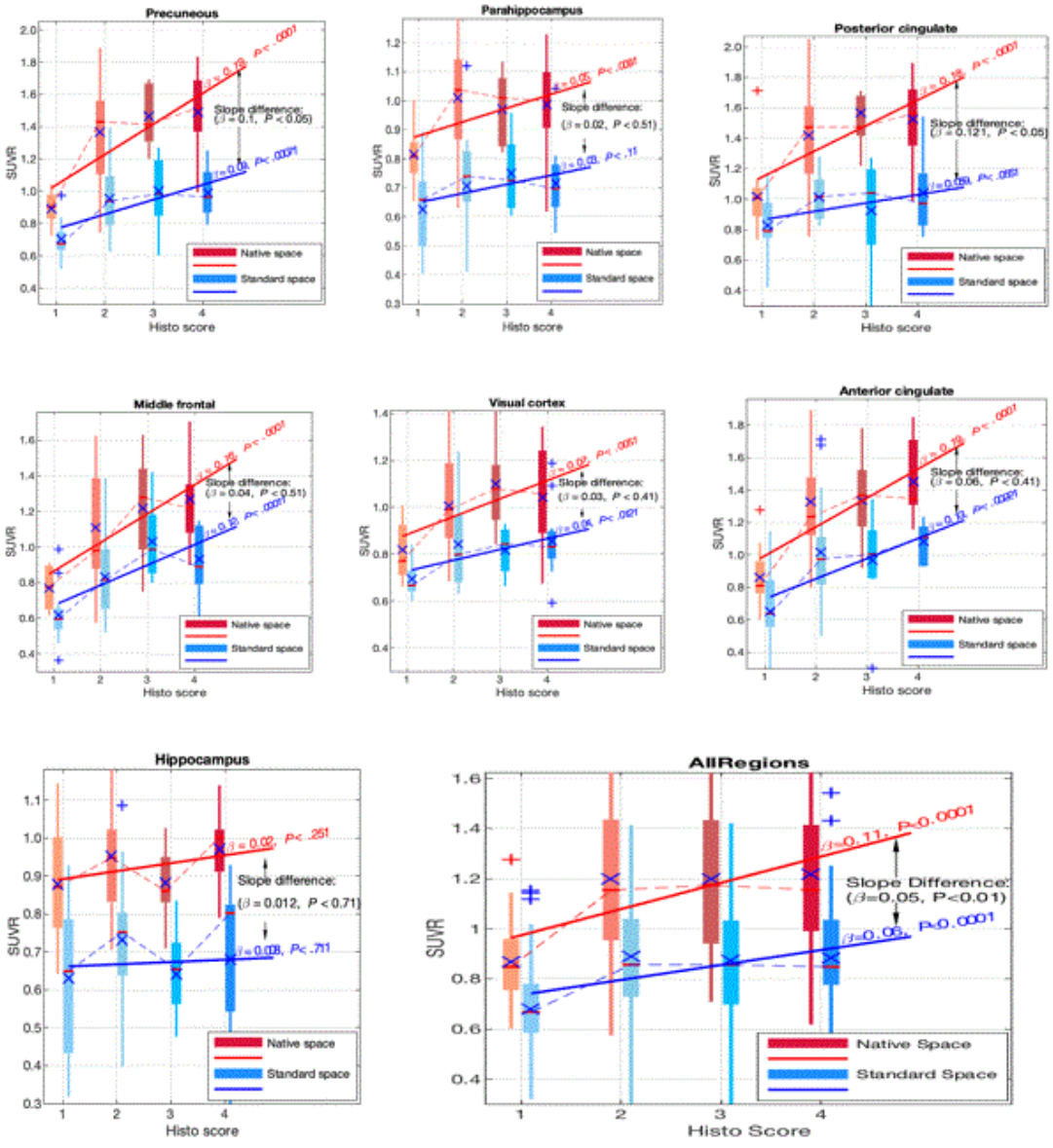


Figure 2. Correlation and slope test parameters showing SUVR distribution obtained using Native and standard methods based on postmortem histopathological staging.

Disclosures: **M. Tahmi:** None. **W. BouZeid:** None. **Q.R. Razlighi:** None.

Poster

042. Alzheimer's Disease and Other Dementias: Imaging Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 042.22/C74

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

Support: H2020 EU Program: PANA Project _ Grant Agreement 686009

Title: Pana project: Theragnostic approach for Alzheimer's disease

Authors: M. RODRIGUEZ-PEREZ[1], B. PELAZ[3], P. AGUIAR[2], A. POSADO-FERNANDEZ[1], E. POLO[3], M. ARAMBURU-NUÑEZ[1], L. VAZQUEZ-VAZQUEZ[1], F. CAMPOS[1], A. ALMEIDA[4], J. CASTILLO[1], P. DEL PINO[3], ***T. SOBRINO**[1];
[1]Clin. Neurosciences Res. Lab., [2]Mol. Imaging Group, Hlth. Res. Inst. Santiago de Compostela, Santiago de Compostela, Spain; [3]CIQUS, Univ. de Santiago de Compostela, Santiago de Compostela, Spain; [4]Inst. of Biomed. Res. of Salamanca, Salamanca, Spain

Abstract: Objective: Alzheimer’s disease (AD) is the leading cause of dementia. Currently, there is not an effective method for the early diagnosis of AD. Therefore, there is an urgent need to develop new effective early diagnostic and therapeutic strategies. To defeat this challenge, this study bases its approach on the importance of tau in the early pathophysiological processes of AD. Our strategy was based on two fundamental pillars; on one hand, efforts were focused on multimodal PET/MRI imaging as the best solution for diagnostic purposes, combining the high structural characterization of tissue provided by MRI with the enhanced sensitivity of PET imaging. On the other hand, the challenging development of a theragnostic nanostructure was focused on tau detection, which has to deliver theragnostic agents into the brain to provide *in situ* diagnostic and therapeutic effects.
Methods/Results: Our design was based on iron oxide nanoparticles (NPs) doped with zinc and manganese (1), stabilized with oleic acid/oleylamine in organic solvents, which were transferred to the aqueous phase by coating with an amphiphilic polymer (dodecyl-grafted-poly(isobutylene-alt-maleicanhydride, in the followign referred to as PMA) (2, 3). Modified-PMA conferred colloidal stability to the NPs in high ionic strength media and provides several chemical groups (e.g., carboxyl, dibenzocyclooctyne, furfuryl, etc.) for functionalization with other macromolecules, including homing antibodies (anti-tau and anti-B_amyloid oligomers), polyethylene glycol, PET radiotracers (Zirconium-^{89}) and fluorescence markers. The designed nanostructures worked as good contrast agents for MRI in T2 and T2* sequences (r^2=409.1 mM^{-1} s^{-1}). Furthermore, non-toxic effects (up to concentrations of [Fe] greater than 50 ug/mL) were observed *in vitro* in both endothelial (bEnd.3) and primary neurons cell cultures by LDH, MTT, and IP/Annexin. Effective biofunctionalization of NPs with monoclonal antibodies of tau and B-amyloid was obtained by the click-chemistry cu-free method. Radiolabelling of NPs for PET

studies was performed with ^{89}Zr.
Conclusions: This study shows a novel theragnostic nanostructures that specifically recognize very-early molecular markers of AD, and can be detected by means of non-invasive imaging methodologies (MRI and/or PET, which are already common techniques accessible in most hospitals), and eventually provide a therapeutic action if needed.
References: [1]. J. Jang et al., Angew. Chem. Int. Ed. 2009, 48, 1234-1238. [2]. Q. Zhang, et al., Chem. Mater. 2015, 27 (21), 7380-7387. [3]. B. Pelaz, et al., ACS Nano 2015, 9 (7), 6996-7008.

Disclosures: **M. Rodriguez-Perez:** None. **B. Pelaz:** None. **P. Aguiar:** None. **A. Posado-Fernandez:** None. **E. Polo:** None. **M. Aramburu-Nuñez:** None. **L. Vazquez-Vazquez:** None. **F. Campos:** None. **A. Almeida:** None. **J. Castillo:** None. **P. del Pino:** None. **T. Sobrino:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.01/C75

Topic: C.03. Parkinson’s Disease

Title: Parkinson's disease-related phenotype characterization of A53T alpha-synuclein IPSC-derived dopaminergic cultures

Authors: T. FERRARO, R. REMELLI, E. BIANCHINI, E. TORCHIO, A. TOTI, C. GRIFFANTE, *M. CORSI;
In Vitro Pharmacol., Aptuit (Verona) Srl, an Evotec Co., Verona, Italy

Abstract: Introduction: Parkinson disease (PD) is a progressive neurological disease caused by selective loss of dopaminergic (DA) neurons in the substantia nigra. Although the majority of PD cases are sporadic, familial PD mutations provide a valuable tool for understanding and modelling basic pathophysiological mechanisms. We used MyCell® DopaNeurons A53T carrying the A53T mutation in the SNCA gene and healthy isogenic control iCell® DopaNeurons (FujiFilm) to investigate disease-relevant phenotypes including alpha synuclein (αSyn) accumulation, calcium dysregulation and mitochondrial dysfunction.
Methods and Results: Cell culture and neurons differentiation was carried out in 384-well plate format. High content imaging studies revealed that more than 70% of viable neurons were tyrosine hydroxylase-positive at day 14 post-seeding in both A53T and control DA cultures, with comparable viability up to day 28. Since A53T mutation is reported to induce αSyn protein accumulation and aggregation, we sought to determine whether the A53T culture recapitulates this phenotype using Meso Scale Discovery®, a highly sensitive and quantitative technology. Results revealed a time-dependent selective accumulation of αSyn in A53T cultures, with a maximal difference between the two cultures detected at day 28 (1.61 ±0.18 folds, three

independent cultures). Furthermore, spontaneous calcium oscillations were studied by applying FLIPR® technology. A different pattern of calcium oscillations was observed between A53T and control mature cultures. A53T neurons displayed 1.5-fold higher peak frequency and average peak amplitude compared to controls, suggesting a dysregulation of intracellular calcium homeostasis, being associated with mitochondrial oxidative stress. Mitochondrial membrane potential (MMP) was used as a read-out of possible mitochondrial dysfunction. A high content imaging assay combining cell viability and MMP quantification showed a 10% to 20% higher depolarization of mitochondria in A53T cultures compared to controls at different time-points (day 28-35).
Conclusions: Key cellular features linked to PD pathogenesis were identified in A53T iPSC-derived DA cultures. Based on this phenotypic characterization, an array of different assays was developed and will provide a valuable tool for the identification of neuroprotective compounds in PD drug discovery programs.

Disclosures: T. Ferraro: None. **R. Remelli:** None. **E. Bianchini:** None. **E. Torchio:** None. **A. Toti:** None. **C. Griffante:** None. **M. Corsi:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.02/C76

Topic: C.03. Parkinson’s Disease

Title: Role of the indirect pathway in L-Dopa-induced dyskinesia

Authors: *L. ANDREOLI[1], J. JAKOBSSON[3], M. A. CENCI[2];
[1]Exptl. Med. Sci., [2]Dept. of Exptl. Med. Sci., Lund Univ., Lund, Sweden; [3]Wallenberg Neurosci. Ctr., Lund, Sweden

Abstract: Background: L-DOPA-induced dyskinesia (LID) is a major complication of the treatment of Parkinson’s disease (PD). Current theories attribute LID to excessive stimulation of dopamine D1 receptors (D1Rs) on direct pathway spiny projection neurons (dSPNs), but very little is known about the specific role of indirect pathway neurons (iSPNs), which express D2 receptors (D2Rs).
Aim: To determine whether changes in iSPN activity affect dyskinetic behaviors in a mouse model of PD using a combination of chemogenetic and pharmacological tools.
Methods: Adora2a-Cre mice sustained unilateral injections of 6-OHDA in the medial forebrain bundle or sham lesions. Cre-inducible adeno-associated viral vectors (AAVs) coding for hM4Di (a Gi-coupled DREADD) were delivered to the dorsolateral striatum in both intact and 6-OHDA-lesioned mice. Four weeks post AAV delivery, mice underwent tests of forelimb use asymmetry, horizontal and vertical activity, and rotations. Recordings were made after treatment with

clozapine-N-oxide (CNO) or vehicle. Thereafter, mice were sequentially treated with increasing doses of the dopamine (DA) D1 agonist SKF38393 and abnormal involuntary movements (AIMs) were recorded repeatedly after administering SFK38393 alone or combined with CNO.
Results: In both intact and 6-OHDA-lesioned Adora2aCre mice transduced with iSPN-Gi-DREADD, CNO treatment induced a significant increase in contralateral turning behavior. In lesioned mice, CNO restored forelimb use symmetry in the cylinder test, mimicking the therapeutic effect of L-DOPA. Although CNO did not induce AIMs when given alone, it significantly potentiated the dyskinetic action of SKF38393, with a particularly strong effect on axial AIMs.
Conclusions: These results indicate that a Gi-DREADD-mediated inhibition of iSPNs is sufficient to reverse the parkinsonian motor phenotype of 6-OHDA-lesioned mice without inducing dyskinesia. However, Gi-DREADD-mediated iSPN inhibition aggravates dyskinesias that are induced by D1 receptor stimulation, supporting a cooperativity of direct- and indirect pathways in the emergence of LID (Alcacer et al JCI 2017).

Disclosures: **L. Andreoli:** None. **J. Jakobsson:** None. **M.A. Cenci:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.03/C77

Topic: C.03. Parkinson’s Disease

Support: NIH grant NS096032
NIH grant NS101134

Title: MicroRNA-365 promotes cell death by inhibiting IGF-1 pathway

Authors: *J. ZHANG, R. YAN, M. M. MOURADIAN;
Neurology, RWJMS, Rutgers, Piscataway, NJ

Abstract: MicroRNAs (miRNAs) are abundant, endogenous, short, noncoding RNAs that act as important post-transcriptional regulators of gene expression by base-pairing with their target mRNA. Accumulating evidence indicate that miRNAs play an important role in neurodegenerative diseases, including Parkinson’s disease, Alzheimer's disease, and Huntington's disease. The Insulin-like growth factor 1 (IGF-1) signaling pathway serves broad functions including the control of neuronal excitability, nerve cell metabolism, oxidative stress and cell survival. Oxidative stress is induced by an imbalance in the redox state involving either excessive generation of reactive oxygen species or dysfunction of the cell’s antioxidant system, which is a key factor that participates in the pathogenesis of neurodegenerative diseases. Previous studies suggest that oxidative stress inhibits the IGF-1 pathway as one mechanism

leading to cell death, but the factor(s) mediating this pathway is unclear. The present study was carried out to address this knowledge gap using qPCR, MTS assay for cell death, and Western blots in authenticated SH-SY5Y cells. All experiments were performed in triplicates and were repeated three times. We found that miR-365 levels increase significantly when cells are challenged with hydrogen peroxide (H_2O_2) or 1-methyl-4-phenylpyridinium (MPP+). In addition, exogenous overexpression of miR-365 accelerates oxidative stress-induced cell death. Interestingly, addition of IGF-1 to the culture medium mitigates miR-365-facilitated cell death. Consistent with this cell death data, we found that miR-365 inhibits the IGF-1 pathway by down-regulating mTOR and AKT activity, and this inhibition can be reversed by the addition of IGF-1. These results suggest that miR-365 may be an important mediator that links oxidative stress and neuronal death through inhibition of the IGF-1 - mTOR - AKT pathway. These findings raise the possibility that miR-365 provides a target for developing therapeutics designed to slow the progression of neurodegenerative diseases. Support: NIH grants NS096032 and NS101134.

Disclosures: **J. Zhang:** None. **R. Yan:** None. **M.M. Mouradian:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.04/C78

Topic: C.03. Parkinson’s Disease

Support: FWF P27809, W1101
TWF UNI-0404/2345

Title: Role of auxiliary beta-subunits for Cav2.3 calcium channel signaling in dopamine neurons

Authors: *A. SILLER[1], N. T. HOFER[1], K. VILUSIC[1], E.-M. FRITZ[1], T. SCHNEIDER[2], H. J. DRAHEIM[3], J. STRIESSNIG[1], N. J. ORTNER[1];
[1]Dept. of Pharmacol. and Toxicology, Inst. of Pharmacy, CMBI, Univ. of Innsbruck, Innsbruck, Austria; [2]Univ. Cologne, Köln/Cologne D-50931, Germany; [3]Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany

Abstract: Background and Aim:
Voltage-gated Ca^{2+} channels transform membrane depolarizations into intracellular Ca^{2+} signals and are strongly associated with CNS disorders. More recently there is emerging evidence that Cav2.3 R-type Ca^{2+} channels contribute to the high vulnerability of substantia nigra dopamine (SN DA) neurons in Parkinson’s disease. We therefore biophysically characterized the activity of Cav2.3 combined with different auxiliary β-subunits during simulated SN DA tonic pacemaking and phasic burst firing patterns.
Methods:

Cav2.3e stably expressing HEK293 cell lines (including human β3- and α2δ1-subunits) were generated using the Flp-In T-REx system (Invitrogen). Channel properties were investigated by whole cell patch-clamp recordings using 2 or 15 mM Ca^{2+} or Ba^{2+} as charge carriers. Typical SN DA regular action potential waveforms (recorded from a TH^{+} neuron in a mouse brain slice; 2.5 Hz) or simulated burst firing protocols were applied as command voltages and Cav2.3e current was measured. For the investigation of β-subunit modulation of Cav2.3e gating tsA-201 cells were transfected with Cav2.3e α1, α2δ1, eGFP and either β2a or β3.
Results:
During regular SN DA pacemaking firing patterns, Cav2.3e channels inactivated completely within 1 min when β3-subunits were part of the channel complex. In contrast, with β2a-subunits, steady-state inactivation of Cav2.3e shifted by 38 mV to more positive potentials, leading to ~40% remaining current after 5 min of tonic firing. During typical three-spike bursts integrated Cav2.3e I_{Ca} increased about 6-fold with β3- and β2a-subunits respectively. After burst firing induced pauses at hyperpolarized potentials (1.5 s, -82 mV) only Cav2.3e channels in complex with β3-subunits recovered sufficiently from inactivation, leading to a more pronounced increase of I_{Ca} during the first post-pause action potential.
Conclusion:
Our data predict that during typical SN DA firing patterns β2a- but not β3-subunits support continuous Cav2.3 Ca^{2+} inward current. During burst activity Cav2.3 channels associated with β2a or β3-subunits lead to a pronounced increase of Ca^{2+} influx. If confirmed in SN DA neurons, Cav2.3-mediated I_{Ca} could contribute to Ca^{2+}-induced oxidative stress in Parkinson's disease pathology.

Disclosures: A. Siller: None. **N.T. Hofer:** None. **K. Vilusic:** None. **E. Fritz:** None. **T. Schneider:** None. **H.J. Draheim:** None. **J. Striessnig:** None. **N.J. Ortner:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.05/C79

Topic: C.03. Parkinson's Disease

Title: H63D HFE protects cells from alpha-synuclein mediated toxicity in pre-formed fibril model of Parkinson's disease

Authors: *Y. KIM[1], J. R. CONNOR[1], M. STAHL[2];
[1]Dept of Neurosurg., [2]Dept of Neurol., Pennsylvania State Univ., Hershey, PA

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders, affecting more than 7.5 million people worldwide. PD pathology is characterized by the presence of alpha-synuclein-containing Lewy bodies in the dopaminergic neurons of the substantia nigra

(SN). Increased levels of iron in the SN is also a consistent feature of PD; however, it is unclear how these two factors interact with each other to result in neuronal cell death. The goal of our study is to understand the effects of iron on alpha-synuclein (aSyn) protein homeostasis in a genetic model of iron overload. Specifically, we are using a known mutation in the *HFE* gene, H63D. HFE plays a role in regulation of cellular iron uptake through the transferrin receptor (TfR). Mutations in HFE can disrupt its interaction with TfR leading to iron-overload. It is noteworthy that H63D is the most common HFE mutation with 13.5% allele frequency in the U.S. population and has been shown to increase brain iron content. We investigated the effects of increased intracellular iron on aSyn aggregation and clearance using PFFs, which are aSyn fibrils formed *in vitro*. PFF fragments can act as seeds to form aSyn aggregates inside cells and cause cellular toxicity. SH-SY5Y cells expressing H63D HFE had decreased basal aSyn level and when treated with PFFs, showed decreased aSyn aggregation. Mutant HFE cells were also protected from PFF mediated cell toxicity. As autophagy is one of the main protein degradation pathways, the basal level of autophagy was measured. This revealed that H63D HFE expressing cells had increased autophagy. Importantly, treatment with an iron chelator (deferiprone; DFP), which is currently in clinical trials for PD, had differential effects in WT and H63D HFE expressing cells. DFP decreased the autophagic flux in H63D HFE cells and eliminated the observed protection from PFF mediated cell death; while, in WT HFE cells, DFP increased the autophagic flux and protected against PFF mediated toxicity. These results collectively reveal a novel role of intracellular iron as a protective factor in aSyn mediated toxicity and further indicate the importance of considering the *HFE* genotype in PD clinical trials involving iron chelation therapy.

Disclosures: Y. Kim: None. **J.R. Connor:** None. **M. Stahl:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.06/C80

Topic: C.03. Parkinson’s Disease

Support: CIHR Grant 341846

Title: Microglial alterations in the putamen of Parkinsonian monkeys with and without L-Dopa treatment and dyskinesias

Authors: T. DIPAOLO, K. PICARD, C. LECOURS, M.-K. ST-PIERRE, M. BOURQUE, L. GRÉGOIRE, L. CANTIN, *M. PARENT, M.-È. TREMBLAY;
Univ. Laval, Quebec City, QC, Canada

Abstract: Microglia are the brain immune cells that exert crucial physiological roles across the lifespan. Activated microglia, were found in the brains of Parkinson's disease (PD) patients and their presence correlates with damage to dopamine neurons in the nigrostriatal pathway. While neuroinflammatory responses seem to be associated with L-Dopa treatment, it is still unclear whether they are dependent from the dyskinetic outcome of the L-Dopa treatment and are causally linked to L-Dopa-induced dyskinesias (LID). To assess whether their functions are impaired in the pathophysiology of PD, the most common neurodegenerative motor disorder, we characterized their changes in density, morphology, ultrastructure, and degradation activity among the sensorimotor functional territory of the putamen, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) cynomolgus monkeys. A group of MPTP monkeys was also treated with oral L-Dopa for one month. These monkeys developed LID similar to what PD patients experience after only five to ten years of treatment. Using light, confocal and electron microscopy, our results showed alterations of microglial density, morphology and function following MPTP intoxication that were partially normalized with L-Dopa treatment. Microglial density, cell body and arborization areas were increased in the PD monkeys, with these cells showing a more hyperramified morphology, whereas L-Dopa-treated animals presented a microglial phenotype similar to control animals. At the ultrastructural level, microglia appeared healthy, without dilation of the Golgi apparatus and endoplasmic reticulum, among other cellular stress markers, in MPTP monkeys. Nevertheless, microglia displayed a reduced number of phagocytic inclusions in the MPTP group, suggesting impaired degradation activity. Moreover, a decreased immunoreactivity for CD68 -a lysosome-associated glycoprotein- was measured in microglia from MPTP animals treated with L-Dopa. The subcellular localization of CD68 among secondary lysosomes and tertiary residual bodies was also confirmed in microglia by electron microscopy. Taken together, these findings revealed significant microglial phenotypic changes during PD pathophysiology that were partially rescued by L-Dopa treatment.

Disclosures: **T. DiPaolo:** None. **K. Picard:** None. **C. Lecours:** None. **M. St-Pierre:** None. **M. Bourque:** None. **L. Grégoire:** None. **L. Cantin:** None. **M. Parent:** None. **M. Tremblay:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.07/C81

Topic: C.03. Parkinson’s Disease

Support: Program Project Grant from Foundation for Fighting Blindness
NIH/NEI T32 training grant

Title: Characterizing the role of Abtb2b in neuronal health

Authors: *H. J. T. NONARATH, E. M. CLARK, B. A. LINK;
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Abstract: With an aging population, the incidence of neurodegenerative diseases is on the rise. Current therapeutic options for these disorders alleviate symptoms, but do not target the basis of disease, as the underlying mechanisms are not well understood. The identification of novel proteins important for maintaining neuron health can highlight new mechanisms and pathways that may play a role. Toward this goal, mutagenized zebrafish were screened for age-related neuronal stress using a transgene (*gap43*:gfp) which expresses GFP upon axon damage. In this screen, retroviral-mutagenized zebrafish were analyzed at 6 months of age for elevated GFP expression in retinal and other neuronal populations. One gene of interested from the screen was ankyrin repeat and BTB (POZ) domain containing protein 2 (Abtb2b). To validate the screen, CRISPRs were designed to target exon 3 and exon 9, subsequently creating a large deletion in known functional domains. At 6 months of age *abtb2b* homozygous mutants were analyzed for expression of *gap4*3:GFP in the retina and optic nerve. Throughout both the retina and optic nerve Abtb2b mutants present with axon swellings in the retinal ganglion cells, indicating axon stress and confirming an important functional role for Abtb2b in neuronal health. Although Abtb2b has not been widely studied, two publications from the Pahan Lab have indicated Abtb2b may play a role in maintaining proteostasis. To expand upon current knowledge, using our mutant *abtb2b* zebrafish line, we have injected wildtype and aggregation-prone (A53T) a-synuclein under an islet2b neuronal promoter. In our wildtype line the volume of A53T a-synulcein at 2 dpf was greater in comparison to the Abtb2b mutants. However; aggregate localization in mutants lines was significantly greater within the axon projections. Additionally; longitudinal imaging studies performed from 2-3 dpf show that expression of A53T a-synculein enhances neuronal death, with *abtb2b* mutants showing significant sensitivity. Collectively, these results suggest loss of Abtb2b disrupts trafficking and clearance of a-synuclein, enhancing susceptibility to neuronal death. Future studies will be performed to characterize the specific role(s) of Abtb2b in regulating a-synuclein aggregate clearance and maintenance of proteostasis.

Disclosures: H.J.T. Nonarath: None. **E.M. Clark:** None. **B.A. Link:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.08/C82

Topic: C.03. Parkinson’s Disease

Title: Regulation of the alternative splicing of the D3/D3nf receptor isoform: By the D1R-PKA-PTB pathway

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Abstract: It has been reported in the striatal-nigral neurons of the basal ganglia direct pathway, the activation of the D3R potentiate the effect of the activation of D1R. However, in conditions of dopaminergic denervation, the activation of D3R acquires an antagonistic effect on the activation of D1R. In relation to this, also in a state of denervation it has been found that there is a decrease in the expression of D3nf isoform that could be related to switching in the activity of D3R. Interestingly, it has been suggested that the D3nf receptor modulates by dimerization the presence of D3R in the membrane, internalizing it and preventing DR3 from signaling. D3nf is a product of the alternative splicing of D3R and one of the major regulators of alternative splicing is the PTB protein. It has been shown that PTB inhibits the expression of D2s receptor which is an isoform of the D2 receptor. In addition, it has been shown that PKA modulates the activity of PTB by regulating its cytoplasmic-nuclear localization. Finally, in striatal-nigral neurons the main activators of PKA are the D1 receptors and given the homology between D2R and D3R it is suggested that PTB regulates the splicing of D3nf. For this project an in silico study was carried out through the Human splicing Finder program to determine the probable participation of PTB in the regulation of alternative splicing of D3nf. In addition, the drug SCH-23390 (antagonist of type D1 receptors) was used and the striatal expression levels of D3R and D3nf of both the protein and mRNA were determined. Our study shown that in the deleted sequence of D3nf DNA there are motifs for PTB binding, indicating the possible control of splicing by PTB. Also, we found that blockade of PKA activity by blockade of D1R decrease the expression of D3nf isoform without modification of D3R functional isoform. Therefore, we propose a molecular mechanism that explains the molecular events that are triggered in the state of dopaminergic denervation that will make us understand the function of the D3nf isoform in D3R function.

Disclosures: O. Casados-Delgado: None. **A. Avalos-Fuentes:** None. **F. Paz-Bermudez:** None. **H. Cortes-Calleja:** None. **B. Florán-Garduño:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.09/C83

Topic: C.03. Parkinson’s Disease

Support: NRF of Korea 2019R1A2C2007897

Title: Mammalian target of rapamycin complex 1 activated by astrocytic TRPV1 regulates the expression of neurotrophic factors in the MPP^+-lesioned rat model of Parkinson's disease

Authors: *J. BAEK[1], Y. CHUNG[2], W.-H. SHIN[3], B. JIN[2];
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Abstract: We have recently shown that Transient receptor potential vanilloid 1 (TRPV1) on astrocytes mediates production of neurotrophic factors (NTFs) in the MPP^+-lesioned rat model of Parkinson's disease (PD). However, the precise molecular mechanisms are unknown. As mammalian target of rapamycin complex 1 (mTORC1) pathway can regulate the production of NTFs, we hypothesized that it could be involved in TRPV1-mediated production of neurotrophic factors on astrocytes in MPP^+-lesioned rats. MPP^+ increased expression of TRPV1, phosphorylated (p-) mTORC1 signaling molecules (p-p70S6K, p-S6, and p-4EBP1) and NTFs on astrocytes in the substantia nigra (SN) *in vivo*. The selective knockdown of astrocytic TRPV1 attenuated MPP^+-induced increases in levels of p-p70S6K, p-S6, p-4EBP1, and NTFs in the SN. In addition, the selective knockdown of p70S6K or 4EBP1 in astrocytes decreased the levels of NTFs in the MPP^+-lesioned SN, indicating the existence of TRPV1-mTORC1-NTFs signaling pathway. These results suggest that the mTORC1 signaling pathway is involved in the production of NTFs via astrocytic TRPV1 and might be a novel therapeutic target in the treatment of PD.

Disclosures: J. Baek: None. **Y. Chung:** None. **W. Shin:** None. **B. Jin:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.10/C84

Topic: C.03. Parkinson's Disease

Support: MINECO Grant 2016-77541-R
La Caixa Banking Foundation Grant HR17-00513
Parkinson's U.K.

Title: Age-dependent neuromelanin accumulation in a novel humanized transgenic mouse model for Parkinson's disease and brain aging

Authors: A. LAGUNA[1,2], N. PEÑUELAS[1,2], J. ROMERO-GIMÉNEZ[1,2], M. GONZALEZ-SEPULVEDA[1,2], L. MIQUEL-RIO[3], N. BENSENY-CASES[4], B. RODRÍGUEZ-GALVÁN[1,2], T. CUADROS[1,2], E. ÁLVAREZ-MARIMON[5], A. PARENT[1,2], F. CACHO-NERIN[6], I. CARBALLO-CARBAJAL[1,2], J. CLADERA[7], A. BORTOLOZZI[3], *M. VILA[1,2,8,9];
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Neuropharm., IIBB–CSIC, August Pi i Sunyer Biomed. Res. Inst. (IDIBAPS)-Center for Networked Biomed. Res. on Mental Hlth. (CIBERSAM), Barcelona, Spain; [4]ALBA Synchrotron Light Source, Cerdanyola del Vallès, Spain; [5]Dept. of Biochem. and Mol. Biol., Biophysics Unit and Ctr. of Studies in Biophysics-Universitat Autònoma de Barcelona, Bellaterra, Spain; [6]Diamond Light Source, Harwell Sci. and Innovation Campus, Didcot, United Kingdom; [7]Dept. of Biochem. and Mol. Biol., 4Biophysics Unit and Ctr. of Studies in Biophysics, Univ. Autònoma de Barcelona, Bellaterra, Spain; [8]Dept. of Biochem. and Mol. Biol., Autonomous Univ. of Barcelona, Cerdanyola del Vallès, Spain; [9]Catalan Inst. for Res. and Advanced Studies (ICREA), Barcelona, Spain

Abstract: Parkinson's disease (PD) is characterized by a selective and progressive loss of neurons that contain the dark brown pigment neuromelanin (NM), especially neurons from the substantia nigra (SN) and the locus coeruleus (LC), as well as, to a lesser extent, neurons from the ventral tegmental area (VTA) and the dorsal motor nucleus of the vagus nerve (DMNV). In humans, NM accumulates with age, the latter being the main risk factor for PD. The contribution of NM to PD pathogenesis remained unknown because, unlike humans, common laboratory animals lack NM. To overcome this major limitation, we have recently generated a new humanized rodent model based on the overexpression of human tyrosinase in the SN (AAV-hTyr). These animals show an age-dependent production of human-like NM, up to levels reached in elderly humans, and an age-dependent parkinsonian phenotype. We have now generated a humanized transgenic mouse model (Tg-TH-hTyr) that represents the first model that recapitulates the age-dependent accumulation and distribution of NM in all catecholaminergic neuronal cell groups in the brain, including SN, VTA, LC, and DNV. Using this unique animal model, we have assessed the functional, morphological and molecular implications of progressive NM accumulation in cellular functions. Our results show that NM accumulation affects neuronal function and viability in different catecholaminergic neuronal groups, which is relevant to PD pathogenesis and to the manifestation of both motor and non-motor symptoms of the disease.

Disclosures: A. Laguna: None. **N. Peñuelas:** None. **J. Romero-Giménez:** None. **M. Gonzalez-Sepulveda:** None. **L. Miquel-Rio:** None. **N. Benseny-Cases:** None. **B. Rodríguez-Galván:** None. **T. Cuadros:** None. **E. Álvarez-Marimon:** None. **A. Parent:** None. **F. Cacho-Nerin:** None. **I. Carballo-Carbajal:** None. **J. Cladera:** None. **A. Bortolozzi:** None. **M. Vila:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.11/C85

Topic: C.03. Parkinson’s Disease

Support: NSF Grant DGE1252522

Title: Cell type and brain region-specific chromatin abnormalities in dopamine depleted mice

Authors: *A. J. LAWLER[1], A. R. BROWN[1], R. S. BOUCHARD[2], I. M. KAPLOW[1], N. TOONG[1], C. SRINIVASAN[1], Y. KIM[1], N. SHIN[1], A. H. GITTIS[2], A. R. PFENNING[1]; [1]Computat. Biol., [2]Biol. Sci., CARNEGIE MELLON UNIVERSITY, Pittsburgh, PA

Abstract: Neuron subtype dysfunction is a key contributor to the motor deficits observed in dopamine depleted mouse models of Parkinson's disease. Specifically, parvalbumin-expressing (PV+) neurons in the external globus pallidus (GPe) spike less frequently in the dopamine depleted state and cell type-specific optogenetic stimulation of these neurons—but not indiscriminate GPe stimulation—rescues normal motor behavior [1]. Yet, the molecular properties underlying these electrophysiological changes remain unknown. We developed a new viral affinity purification method, based on INTACT, to isolate PV+ and PV- nuclei from the mouse brain. Applying this technology, we performed targeted assessments of the cell type-specific epigenetic effects of dopamine depletion within three brain regions: GPe, striatum, and isocortex. Using the Assay for Transposase-Accessible Chromatin (ATAC-seq), we identified hundreds of regional open chromatin changes in PV+ and PV- cell types after depletion. Affected sites are enriched within areas of the genome related to protein metabolism and translation, implicating these processes in the consequences of dopamine depletion. Additionally, we characterize region and cell type-specific open chromatin regions containing Parkinson's disease-associated variants, connecting potential disease mechanisms through genetic predisposition to gene regulation to pathophysiology. These results provide new insight into the molecular progression of Parkinson's disease at the resolution of individual cell types and tissues. Moreover, they initiate new candidates for gene therapy targets in patients.
1. Mastro, K. J. *et al.* Cell-Specific Pallidal Intervention Induces Long-Lasting Motor Recovery in Dopamine Depleted Mice. *Nat. Neurosci.* **20,** 815-823 (2017).

Disclosures: A.J. Lawler: None. **A.R. Brown:** None. **R.S. Bouchard:** None. **I.M. Kaplow:** None. **N. Toong:** None. **C. Srinivasan:** None. **Y. Kim:** None. **N. Shin:** None. **A.H. Gittis:** None. **A.R. Pfenning:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.12/C86

Topic: C.03. Parkinson's Disease

Support: Carver Chair of Neuroscience

Title: Investigating the role of a Prkar1b mutation previously associated with a rare neurodegenerative disorder

Authors: *M. E. GAINE[1], M. ARGUE[1], R. ILOUZ[2], T. ABEL[1];
[1]Iowa Neurosci. Inst., Univ. of Iowa, Iowa City, IA; [2]The Azrieli Fac. of Med., Bar Ilan Univ., Ramat Gan, Israel

Abstract: The Protein Kinase CAMP-Dependent Type I Regulatory Subunit Beta (*Prkar1b*) gene encodes a regulatory subunit of cyclic AMP-dependent protein kinase A (PKA). Within the first coding exon of this gene, a heterozygous missense mutation was identified in a family with FUS-negative neuronal intermediate filament inclusion disease (NIFID). The affected family members showed behavioral changes including motor deficits, increased anxiety and memory loss and, therefore, we wanted to study the impact of this mutation in different behavioral paradigms. To do this, we replicated the mutation in a mouse model using CRISPR/Cas9 technology and performed both behavioral and molecular testing. We chose to initially study the potential motor deficits in this mouse model using Rotarod testing. Because the human phenotype was seen at age 45-64, we tested a wide range of ages (2months-17 months old). There was no overall difference between the groups (*Prkar1b*$^{+/-}$ N=24, Wildtype N=32; mixed sex cohort), although a trend of decreased latency was seen in *Prkar1b* mice, especially in mice between 2-10 months old. Notably, when we correlated age with latency during the trial with the most significant difference, we found that wildtype mice had a negative correlation between age and latency to fall (R^2=0.254, P=0.02), which was not seen in *Prkar1b* mice (R^2=0.016, P=0.66). This suggests that wildtype mice lose motor coordination as they get older, but *Prkar1b* mice consistently perform poorly, regardless of age. On the molecular level, the presence of the mutation has been predicted to be damaging (PolyPhen-2) and to alter splice sites (MutationTaster). Therefore, we used Q-PCR to quantify *Prkar1b* gene expression and confirm the functional relevance. In the cerebellum, hippocampus, and striatum, *Prkar1b* expression was significantly down-regulated in the presence of the mutation. None of the other subunits (*Prkar1a*, *Prkar2a*, and *Prkar2b*) were differentially expressed in our mouse model, suggesting that there is no compensatory mechanism following the down-regulation of *Prkar1b*. Further work has begun to investigate anxiety behavior and memory deficits in our mouse model. Complete characterization will further our understanding of this gene and its role in neurodegenerative disorders.

Disclosures: **M.E. Gaine:** None. **M. Argue:** None. **R. Ilouz:** None. **T. Abel:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.13/C87

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS 1R01NS092667
NIH/NIA R01 AG060195
Harold and Ronna Cooper Family
The Orchard Foundation
The Consolidated Anti-Aging Foundation

Title: Cellular mechanisms involved in the subset distribution of lower GBA activity in idiopathic Parkinson's disease

Authors: *P. HALLETT, E. MOLONEY, R. THOMAS, O. ISACSON;
Neuroregeneration Inst., Harvard Med. Sch. / McLean Hos., Belmont, MA

Abstract: Previous work in our lab determined that a subset of idiopathic Parkinson's disease (PD) patient fibroblasts phenocopied mitochondrial vulnerability observed in mutant LRRK2 PD fibroblasts (Smith G *et al.*, 2016, Mol. Neurobiol.). We have previously shown that glucocerebrosidase (GCase) activity progressively declines with age, and is decreased in PD patient substantia nigra compared to healthy patients (Rocha E *et al*., 2015, Ann Clin Transl Neurol). To determine if PD patient fibroblasts could be stratified based on GCase activity, we used a large cohort of idiopathic PD-patient fibroblasts and measured basal levels of lysosomal GCase activity. Healthy subject-derived fibroblasts (n=14), idiopathic Parkinson's disease (PD) patient (n=32), and mutant *GBA1* PD patient fibroblasts (n=8) lines were obtained from Coriell, and NINDS repositories. Idiopathic PD patient lines were sequenced to confirm the absence of mutations in *GBA1* and *LIMP-2* genes. GCase activity was diminished in a subset of idiopathic PD-patient fibroblasts as determined by a Gaussian model. This mathematical modeling showed a significant bimodal distribution of GCase activity in human-derived fibroblasts, with approximately 35% of the cases at normal GCase activity compared to controls, and a separate group with approximately 50% GCase activity decline. Measurements of GBA mRNA or protein expression did not correlate with the GCase activity. We therefore explored LIMP-2 transporter levels, post-ER/ER distribution of GBA, and chaperone functions. Our findings will potentially help define the underlying mechanisms for subsets of patients that are vulnerable to glycolipid induced Parkinson's disease, in order to provide appropriate treatments aimed at causality.

Disclosures: P. Hallett: None. **E. Moloney:** None. **R. Thomas:** None. **O. Isacson:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.14/C88

Topic: C.03. Parkinson's Disease

Support: R01NS100090
R01NS088206
R01ES027245
Eugene and Linda Lloyd Endowment and Armbrust Endowment

Title: Trained innate immunity in microglia in response to environmental stress: Relevance to persistent neuroinflammation in Parkinson's disease

Authors: *M. HUANG, J. LUO, S. SARKAR, E. MALOVIC, A. CHARLI, J. HUA, V. ANANTHARAM, A. KANTHASAMY, A. G. KANTHASAMY;
Biomed. Sci., Iowa State Univ., Ames, IA

Abstract: Persistent neuroinflammation mediated by microglia, the innate immune cells of the brain, is a pathophysiological hallmark of many neurodegenerative diseases, including Parkinson's disease (PD). Interestingly, a new concept termed 'trained innate immunity' has recently emerged that refers to the ability of innate immune cells to form long-lasting memories of environmental inflammatory stimuli enabling them to make a heightened response to a secondary inflammatory insult by epigenetic reprogramming. As an environmental inflammatory stimulus, manganese (Mn) exposure has been linked to Parkinsonism in humans. Herein, we explored this novel concept of microglia-mediated trained immunity using LPS as a memory priming trigger and Mn as the secondary environmental trigger. In our study, we first performed qRT-PCR to examine the inflammatory response in an LPS-trained mouse microglia cell (MMC) line and primary mouse microglia (PMG). LPS-trained MMCs and PMGs both displayed a significant increase in the mRNA expression of IL1β, NLRP3, NOS2, IL1α, IL6, IL12β, and TNFα in response to the secondary Mn insult. Notably, Mn-treated, LPS-trained MMCs exhibited an exaggerated inflammatory response as revealed by increased inflammatory cytokine levels. Additionally, iNOS activation as measured by nitrite release was consistent with the qRT-PCR and cytokine assay. These data indicate that Mn exposure triggers trained microglia to recall the previous encounter and stimulates a heightened inflammatory response. We next assessed whether Mn induces trained immunity in microglia through epigenetic reprogramming. Our immunocytochemical studies of Mn-exposed, LPS-trained MMCs show enhanced deposition of the key epigenetic marker of trained immunity, H3K27 acetylation (H3K27ac), together with significant morphological changes. Regarding clinical translation, we probed substantia nigra tissues from human PD brains and aged-match controls with H3K27ac. Interestingly, PD brains showed more H3K27ac accumulation than control brains, further supporting our mechanistic studies. Collectively, these results demonstrate that environmental exposure to neurotoxic stress can activate trained immunity in microglia by triggering the recall of a previous stimulus, leading to a persistent inflammatory response and neurodegeneration. Support: R01NS100090, R01NS088206, and R01ES027245, Eugene and Linda Lloyd Endowment and Armbrust Endowment.

Disclosures: M. Huang: None. **J. Luo:** None. **S. Sarkar:** None. **E. Malovic:** None. **A. Charli:** None. **J. Hua:** None. **V. Anantharam:** None. **A. Kanthasamy:** None. **A.G. Kanthasamy:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.15/C89

Topic: C.03. Parkinson’s Disease

Support: Micheal J. Fox Foundation for Parkinson's Research
NIH R37 NS096241
NIH R01 NS076054
NIH T32NS041234

Title: Examining the role of glucocerebrosidase in LRRK2 mediated Parkinson's disease pathogenesis

Authors: *D. YSSELSTEIN, D. KRAINC;
Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Parkinson’s disease (PD) is a common neurodegenerative movement disorder that affects millions of individuals worldwide. Mutations in the gene encoding leucine rich repeat kinase 2 (LRRK2) are the most common genetic cause of PD, and mutations in the gene encoding glucocerebrosidase (GCase) are the most common genetic risk factor for PD. The precise mechanism through which mutations in these genes lead to PD pathogenesis is unclear. Recent observations by us and others have identified patients with concurrent mutations in LRRK2 and GCase that develop PD at an earlier age than patients with just a single mutation. This observation highlights the possibility of a mechanistic convergence of GCase and LRRK2 mutations in PD pathogenesis. To study a possible mechanistic role of GCase in LRRK2-mediated PD pathogenesis we used induced pluripotent stem cells (iPSCs) reprogrammed from patients with LRRK2 mutations. We differentiated these iPSCs into midbrain dopaminergic neurons and examined the effect of different LRRK2 mutations on lysosomal GCase activity. We found that neurons containing LRRK2 G2019S or R1441C mutations display reduced lysosomal GCase activity relative to neurons derived from isogenic corrected controls and neurons differentiated from healthy control patients. Treatment of these LRRK2 mutant neurons with LRRK2 kinase inhibitors significantly increased lysosomal GCase activity to levels observed in the isogenic control lines and rescued PD related phenotypes in these cells. Additionally, we examined neurons with common GCase mutations which are known to have significantly reduced GCase activity. We found that treatment of these neurons with a LRRK2 kinase inhibitor also led to a significant increase in lysosomal GCase activity which was sufficient to rescue PD related phenotypes in these neurons. These results highlight a role for LRRK2 in the regulation of GCase activity in human dopaminergic neurons. These observations also suggest

that emerging therapies targeted towards LRRK2 inhibition or GCase activation could also have therapeutic benefits in a broader population of PD patients.

Disclosures: D. Ysselstein: None. **D. Krainc:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.16/C90

Topic: C.03. Parkinson's Disease

Title: The effect of chlorinated solvent trichloroethylene on tyrosine hydroxylase 1 expression in zebrafish embryos using qPCR

Authors: *K. D. MCCARTHY[1], P. KUHN[1], B. L. DEL MORAL[2];
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Abstract: Trichloroethylene (TCE) is a common industrial solvent and environmental contaminant that has been linked to an increased prevalence of Parkinson's disease. Parkinson's disease is characterized by marked degeneration of dopaminergic neurons in the midbrain substantia nigra. Despite a proposed toxicological mechanism for TCE-induced neurodegeneration and studies that indicate a connection between chlorinated solvent exposure and Parkinson's disease, evidence of dopamine depletion caused by chlorinated solvents is lacking in zebrafish (*Danio rerio*). This study seeks to develop a zebrafish model for solvent-induced dopamine loss by measuring transcript levels of tyrosine hydroxylase 1 (TH1), the rate-limiting enzyme in the synthesis pathway that produces dopamine. To investigate the effects of TCE on dopamine-containing cells using zebrafish embryos, qPCR was used to measure TH1 in the zebrafish central nervous system during its initial development. Zebrafish embryos were exposed to 10 mM TCE beginning 8 hours post-fertilization until 5 days post-fertilization and then prepared for qPCR using two DNA primers specific to TH1. The effects of these solvents on zebrafish expression of TH1 are compared to a vehicle control and a positive control of 800 μM 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is an established neurotoxin that depletes dopamine-cells and leads to a loss of tyrosine hydroxylase. Future studies will assess the effects of TCE on locomotor activity using behavioral measurements of spontaneous movement and startle response in order to demonstrate that chlorinated solvents affect motor systems via tyrosine hydroxylase depletion and further validate zebrafish as a model organism for investigating the neurodegeneration caused by environmental and industrial toxins.

Disclosures: K.D. McCarthy: None. **P. Kuhn:** None. **B.L. Del Moral:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.17/C91

Topic: C.03. Parkinson’s Disease

Support: NINDS R01NS085155-02

Title: Elucidating the contribution of the inflammatory regulators interleukin 13 and its receptor alpha-1 in Parkinson's disease

Authors: *C. A. AGUIRRE, M. SANCHEZ-ALAVEZ, R. CINTRON-COLON, B. CONTI; Mol. Med., The Scripps Res. Inst., La Jolla, CA

Abstract: Parkinson’s disease (PD) is the second most common neurodegenerative disorder, affecting approximately 1% of the population over the age of 60, and it is primarily characterized by the loss of dopaminergic (DA) neurons of the *substantia nigra pars compacta* (SNc). Although monogenic forms of PD have been identified, most PD cases are believed to be the result of a combination of environmental and genetic factors. Recently, inflammation and its mediators have been proposed to contribute to the neuronal loss that occurs in PD. Thus, inflammatory regulators could be involved in the loss of DA neurons and play a significant role in the onset and/or progression of PD. Therefore, we asked whether cytokines that regulate inflammation may be relevant targets of PD. We collected considerable evidence that two such genes are those encoding for interleukin-13 and for its receptor alpha-1 (IL-13Rα1). We propose to test the hypothesis that these inflammatory regulators contribute to the loss of DA neurons and thereby play a significant role in the development and/or progression of PD. In order to explicate the contribution of IL-13 and IL-13Rα1 to DA neuron loss in PD, we used CRISPR/Cas9 in both *in vitro* and *in vivo* experiments to determine the biological function of a rare single nucleotide polymorphism (SNP) in the human *IL13* gene and a rare SNP in the human *IL13RA1* gene that we found to be associated with PD (early onset for *IL13* SNP). We are determining the biological activity of the mouse homologue of IL-13 SNP and of the mouse homologue of 13Rα1. We compared the effects of chronic peripheral inflammation with bacterial lipopolysaccharide (LPS), a mouse model previously shown to induce neuroinflammation, oxidative stress, and the eventual loss of DA neurons, on behavioral tests in mice homozygous for the rare SNP in the *IL13* gene (13LP mice), mice homozygous for the rare SNP in the *IL13RA1* gene (13RLF mice), and their wildtype littermates. In the parallel rod test a main group effect ($F(1, 26)=18.243$, $p<0.001$ vs. vehicle, n = 5 per group) revealed that mice under LPS treatment had significantly greater number of foot slips. In the pole test a main group effect ($F(1,26)=10.188$, $p<0.004$ vs. vehicle, n = 5 per group) revealed that mice under LPS treatment had significantly lower scores.

Data from our experiments will validate the contribution of IL-13 and IL-13Rα1 to DA neuron loss in PD and reveal them as novel targets for the treatment of PD.

Disclosures: C.A. Aguirre: None. **M. Sanchez-Alavez:** None. **R. Cintron-Colon:** None. **B. Conti:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.18/C92

Topic: C.03. Parkinson's Disease

Support: France Parkinson
Fondation de France
ANR-13-BSV1- 0013-02
ANR TIMMS
FRM,DEQ 20140329552
ANR 2010 MIDI 00801

Title: Microglial glucocorticoid receptors contribution to degeneration of midbrain dopamine neurons and novel insight of TLR9 implication

Authors: *A.-C. COMPAGNION[1], L. MAATOUK[1], M.-A. CARRILLO-DE SAUVAGE[2], A.-P. BEMELMANS[2], R. M. RANSOHOFF[3], F. TRONCHE[1], B. MANOURY[4], S. VYAS[1]; [1]IBPS UPMC, Paris, France; [2]CEA, Fontenay Aux Roses, France; [3]Biogen, Cambridge, MA; [4]INEM Hôpital Necker, Paris, France

Abstract: Inflammation is a one of the major characteristic of Parkinson's disease (PD). We previously showed a critical role of microglial glucocorticoid receptor (GR) in a neurotoxin mouse model of PD, induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injection. Herein we show that microglial GR has a protective role against human A53T-alpha-synuclein-induced dopaminergic neuronal degeneration. In addition, our analysis of GR in microglia of substantia nigra of PD brain samples revealed decreased microglial GR expression indicating that microglial GR plays a role in Parkinson's disease pathology. We had previously reported increased levels of immune Toll-Like Receptor 9 (TLR9) in the striatum of human PD brain samples compared to controls. In this study, we examined the role of TLR9 and its regulation by GRs in degeneration of substantia nigra dopamine neurons. TLR9 agonist, CpG-ODN, induced DN degeneration in mice invalidated for microglial GR and not in control mice. Furthermore, TLR9 knock-down reduced DN loss in the MPTP model. GR also regulates TLR9 activation during MPTP neurotoxicity because TLR9 antagonist suppressed the increased dopaminergic neuronal loss observed in microglia/macrophage GR mutant mice. Mitochondrial

DNA, which is the endogenous ligand to TLR9, also triggered dopaminergic neuronal loss following intra-nigral injection. We additionally explored novel mechanisms by which GR regulates TLR9 activity: GR absence in microglia enhanced TLR9 translocation to endolysosomes and facilitated its cleavage leading to pro-inflammatory gene expression. Overall our work shows that reduced microglial GR activity in SN can stimulate TLR9 activation and DN loss in PD pathology. This study could lead to a potential protective new medicine tackling TLR9 activity.

Disclosures: A. Compagnion: None. **L. Maatouk:** None. **M. Carrillo-de Sauvage:** None. **A. Bemelmans:** None. **R.M. Ransohoff:** None. **F. Tronche:** None. **S. Vyas:** None. **B. Manoury:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.19/D1

Topic: C.03. Parkinson’s Disease

Support: NIH R37 NS096241

Title: Deciphering the role of the P5 type ATPase ATP13A2 in autophagy and calcium homeostasis in long term human neuronal cultures

Authors: *G. MINAKAKI, J. BLANZ, K. OEVEL, C. VALDEZ, K. TRAJKOVIC, D. KRAINC;
Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: The P5 type ATPase 13A2 (ATP13A2) is genetically linked to neurodegenerative disorders, including a rare form of autosomal recessive juvenile-onset parkinsonism with pyramidal degeneration and dementia termed Kufor-Rakeb syndrome, and hereditary spastic paraplegia. While the specific substrates of ATP13A2 have not been identified, the protein has been proposed to transport inorganic cations, polyamines or lipids. Previous studies showed that ATP13A2 exerts a modulatory role on autophagy initiation and clearance via the endolysosomal compartment *(Dehay, Ramirez et al. 2012, Usenovic, Tresse et al. 2012, Bento, Ashkenazi et al. 2016)*. Furthermore, ATP13A2 has been implicated in the regulation of Ca^{2+} levels in the cytosol and endolysosomal compartment *(Ramonet, Podhajska et al. 2012, Narayanaswamy, Chakraborty et al. 2019)*, yet whether this can be attributed to the ATPase function and directly related to autophagy modulation, remains unresolved. The overarching aim of the present study is to address whether ATP13A2 physiologically modulates autophagy via Ca^{2+} transport across endolysosomal membranes, and to elucidate how this may be linked to cortical and dopaminergic neuronal vulnerability. To this end we have established mammalian cell lines stably

overexpressing untagged ATP13A2. In line with previous reports using transient overexpression, we find that ATP13A2 extensively localizes on the endolysosomal compartment, which is known to also serve as a Ca^{2+} store. Preliminary analyses using flow cytometry support that ATP13A2 overexpression may influence cytosolic Ca^{2+} levels at baseline, paralleled by alterations in mTOR signaling and cell morphology. In order to study implications for human neurons, using CRISPR/Cas9 technology we have generated ATP13A2 knockout and isogenic control human iPSC lines, which will be differentiated to dopaminergic and cortical neurons. In these human neuronal cultures we will characterize how loss of ATP13A2 impacts mTOR signaling and neuronal morphology, in parallel with Ca^{2+} levels in the cytoplasm and within endolysosomes. This study will provide novel insight into the physiological role of ATP13A2 with direct implications for human neuron vulnerability in neurodegeneration.

Disclosures: G. Minakaki: None. **J. Blanz:** None. **K. Oevel:** None. **C. Valdez:** None. **K. Trajkovic:** None. **D. Krainc:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.20/D2

Topic: C.03. Parkinson’s Disease

Support: Appel Institute for Alzheimer’s Disease

Title: Cell culture model linking Parkinson’s disease and melanoma

Authors: *A. BOSE[1], D. ELIEZER[2], G. PETSKO[1];
[1]Brigham and Women’s Hosp., Boston, MA; [2]Biochem., Weill Cornell Med., New York, NY

Abstract: Parkinson’s disease is the second most cookies neurodegeneative disease in the world .Melanoma is a malignancy of melanocytes which are the pigment generating cells of the skin. It has been shown that alpha synuclein and melanin co-cocalise in the skin of PD patients.Yasuhiro et al., 2010. We created stable Melanoma cell lines (skmel2) cells and colorectal cell lines (Cacao2) overexpressing WT , A53T, and A30P mutants. We showed that in skmel2 cells alpha synuclein and melanin colocalise in the cytoplasm and in the melanosomes , while in the CaCo2 cells they do not.These results suggest that different α -synuclein mutants have different localizations depending on their membrane binding abilities.Co-localization of α- synuclein and melanin in the melanosomes of the the SK-MEL-2 cells suggest that there may be common signaling pathways that can explain the comorbidity between PD and melanoma.Also, since α-synuclein does not localize in melanosomes in the CACO2 we hypothesize that the effects seen in the SK- cells can be due to α-synuclein.Recently autophagy has also been co related with tumor . Autophagy is down regulated in melanoma(Miracco et al., 2010). However a recent

study has shown that melanoma cells exhibit high levels of autophagy (Lazova et al., 2010).A30P mutation of alpha synuclein induces autophagy shown by increase in LC3B while A53T mutant does not induce autophagy in Skmel 2 cells. All these results suggest that different alpha synuclein mutants behave differently which results in different functional consequences which could explain why some Parkinson's patients develop melanoma while others do not and vice versa.

Disclosures: **A. Bose:** None. **D. Eliezer:** None. **G. Petsko:** None.

Poster

043. Cellular Mechanisms of Parkinson's Disease I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 043.21/D3

Topic: C.03. Parkinson's Disease

Support: Wesley Medical Research
Advance Queensland Innovation Partnership Grant
Dr Gordon is supported by the Advance Queensland Mid-Career Fellowship

Title: Profiling of systemic inflammasome activation markers in Parkinson's disease

Authors: *K. E. ROPER[1], N. BIRCH[1], H. WOODHOUSE[1], S. MANTOVANI[1], J. O'SULLIVAN[2], R. GORDON[1];
[1]Univ. of Queensland Ctr. For Clin. Res., Brisbane, Australia; [2]Dept. of Neurol., Royal Brisbane and Women's Hosp., Brisbane, Australia

Abstract: Parkinson's disease (PD) is a progressive neurological disease that is characterised by chronic neuroinflammation, dopaminergic degeneration and extensive α-synuclein inclusions in the form of Lewy-bodies. Increased levels of immune and inflammatory markers in peripheral fluids and post-mortem tissues have been identified in PD patients. This peripheral inflammation is closely linked to disease etiology and progression. However, the relationship between neuroinflammation/degeneration and peripheral inflammation in PD patients, and how they impact on disease incidence and progression, remains unclear. Recently our groups showed that the microglial NLR family pyrin domain containing 3 (NLRP3) inflammasome is a common pathway triggered by both fibrillary α- synuclein and dopaminergic degeneration in PD. Drugs which inhibit inflammasome activation are neuroprotective in multiple disease models. Our team also found elevated caspase-1 levels in PD patient serum, a key marker of inflammasome activation. Therefore, we sought to comprehensively characterise inflammatory markers associated with NLRP3 activation in peripheral biofluids (blood fractions, urine, and saliva) in patients with PD and compare them to gender and age-matched healthy controls. Our findings suggest that protein-level inflammasome activation markers (ie Caspase-1, IL-1b, ASC) are

differentially expressed in peripheral blood mononuclear cells (PBMCs) of PD patients compared to healthy controls; however, these changes were not detected in accompanying plasma samples. Further, these same markers are mostly undetectable in urine. RNA-based profiling via PCR arrays also identifies a number of transcript level changes in genes of the inflammasome pathway in PBMCs from PD patients when compared to matched healthy controls. Furthermore, when PD PBMC populations are subject to *ex vivo* stimulation of inflammasome pathways, they show differential responses to their healthy counterparts. Taken together, these results suggest that inflammasome pathways is evident in PBMCs of PD patients and may be a target for future disease-modifying therapies. Isolation and storage of biofluids for this research has a dual purpose of creating a high- quality biorepository of PD and healthy control biofluids that will support further research into chronic inflammation which underlies several neurological diseases.

Disclosures: K.E. Roper: None. **N. Birch:** None. **H. Woodhouse:** None. **S. Mantovani:** None. **J. O'Sullivan:** None. **R. Gordon:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.01/D4

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: Sanofi

Title: AAV mediated long term reduction of human tau in a tauopathy mouse model

Authors: *B. ELMER[1], B. RICHARDS[2], Z.-Y. YANG[3], G. J. NABEL[3], L. M. STANEK[1], L. S. SHIHABUDDIN[1];
[1]Neurosci., [2]Genomics, Sanofi, Framingham, MA; [3]Sanofi, Cambridge, MA

Abstract: Tauopathies are a set of neurodegenerative diseases characterized by the abnormal accumulation of tau protein within neurons and/or glial cells. Reducing or ablating tau expression in multiple models of neurological disease prevents and reverses neuropathology, slows tau fibril formation and spread, and improves cognitive deficits. RNA interference (RNAi) has shown increasing promise as a therapeutic modality to reduce levels of target mRNA and protein. Here, we have used a combination of approaches to identify and validate RNAi sequences designed to reduce human tau for the treatment of tauopathy.
To maximize the translational potential of individual RNAi sequences, we only selected those with low-off-targeting liability while retaining homology to multiple species. Off-targeting analysis was performed in silico to select sequences with minimal predicted off-targets to the mouse or human transcriptome. Sequences were first tested for knockdown efficiency in vitro

using U2OS cells stably expressing 4R human tau. Tau protein was quantified via ELISA three days post-transfection. Twelve sequences significantly reduced tau protein between 50 to 75% relative to a non-targeting control vector.
Neurodegenerative diseases will likely require treatment for years, something adeno-associated virus (AAV) gene delivery is well suited to address. Therefore AAV-RNAI tau RNAi vectors were generated for for testing in the Tau22 mouse model of tauopathy. These mice express 1N4R human tau with the FTD-associated G272V and P301S mutations which drive progressive accumulation of tau pathology and subsequent cognitive deficits, making these mice amenable for testing the in vivo efficacy of these vectors. AAVs will be delivered intracranially and allowed to express for 6 weeks before in vivo target reduction is quantified. Sequences will be rank-ordered taking into account in vivo knockdown efficiency and predicted off-target activity. Sequences with the best ranking will be expressed for up to 6 months and mice will be assessed for levels of hyperphosphorylated tau and neurofibrillary tangles, as well as for improvements in cognition at multiple time points. CNS and peripheral biomarkers reflecting target engagement or disease modification will be measured to provide additional preclinical support for the translational potential of a total-tau lowering approach for tauopathies.

Disclosures: **B. Elmer:** A. Employment/Salary (full or part-time):; Sanofi US. **B. Richards:** A. Employment/Salary (full or part-time):; Sanofi US. **Z. Yang:** A. Employment/Salary (full or part-time):; Sanofi US. **G.J. Nabel:** A. Employment/Salary (full or part-time):; Sanofi US. **L.M. Stanek:** A. Employment/Salary (full or part-time):; Sanofi US. **L.S. Shihabuddin:** A. Employment/Salary (full or part-time):; Sanofi US.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.02/D5

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

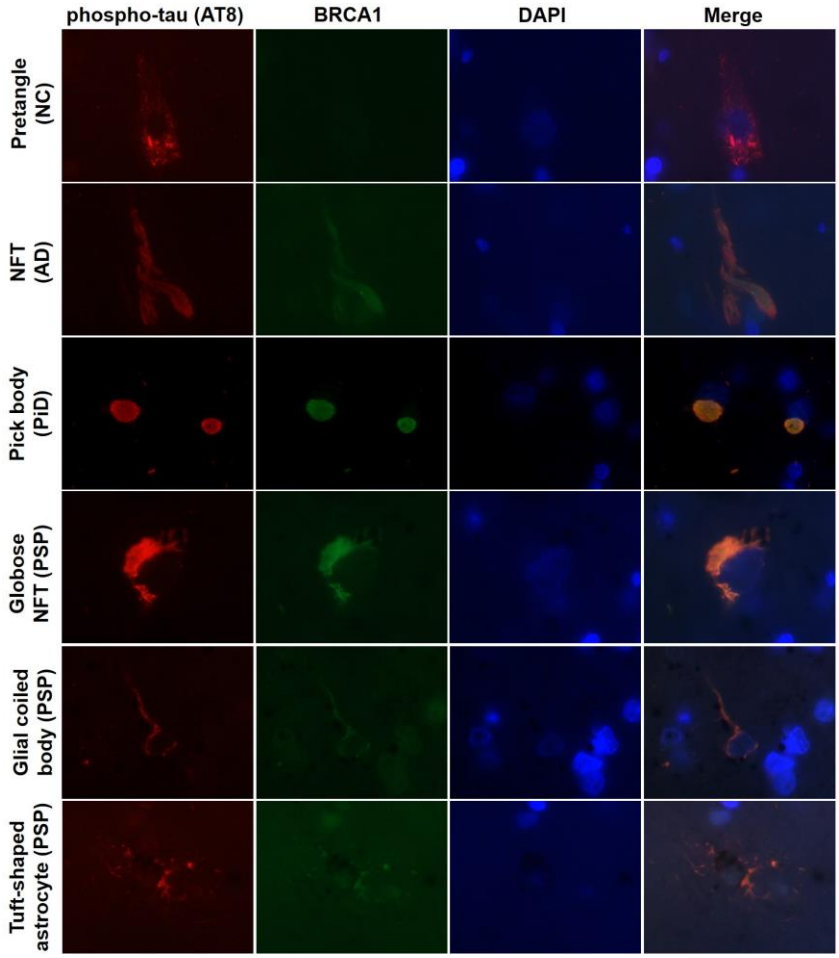
Support: JSPS KAKENHI Grant Number JP19J10924

Title: Mislocalization of DNA repair protein BRCA1 in human tauopathies

Authors: ***M. KURIHARA**[1,2], T. MANO[1], S. MURAYAMA[3], A. IWATA[1], T. TODA[1]; [1]Dept. of Neurol., Grad. Sch. of Medicine, The Univ. of Tokyo, Tokyo, Japan; [2]Res. Fellow, Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Dept. of Neuropathology (the Brain Bank for Aging Research), Tokyo Metropolitan Geriatric Hosp. and Inst. of Gerontology, Tokyo, Japan

Abstract: The mechanism of tau aggregates causing neuronal dysfunction and neuronal loss in tauopathy patients is still controversial. We previously reported that in Alzheimer's disease

(AD), tau aggregation induces mislocalization and coaggregation of DNA repair nuclear protein BRCA1, and that subsequent dysfunction of DNA repair may be an important pathway in AD pathology. However, whether this mislocalization of BRCA1 induced by tau aggregation is also present in other human tauopathies are unknown. The aim of this study was to evaluate whether BRCA1 protein is mislocalized to the cytoplasm and colocalize with tau aggregates in tauopathy patients' brains. We evaluated autopsy brains of 4 Alzheimer's disease (AD), 2 Pick's disease (PiD), 3 progressive supranuclear palsy (PSP), 3 corticobasal degeneration (CBD), 4 normal control (NC), and 4 disease controls (Parkinson's disease with dementia, Lewy body dementia, multiple system atrophy, and amyotrophic lateral sclerosis). Immunohistochemistry was performed using mouse monoclonal antibodies against phosphorylated tau (AT8), BRCA1 (MS110), phosphorylated α-synuclein (pSyn#64), and phosphorylated TDP-43 (pS409/410). Colocalization was confirmed by immunofluorescence double staining. Colocalization of BRCA1 with tau aggregates were seen in neurofibrillary tangles (NFT) and neuropil threads in AD, Pick bodies in PiD, and globose NFT and glial coiled bodies in PSP, but only partially in tuft-shaped astrocytes in PSP, and none in pretangles of NC, pretangles and astrocytic plaques in CBD. Mislocalization of BRCA1 was not seen in disease controls. BRCA1 was mislocalized to the cytoplasm and colocalized with tau aggregates in not only AD but also in PiD, and PSP. Coaggregation of BRCA1 with tau may be also involved in the pathogenesis of PiD and PSP.



Disclosures: **M. Kurihara:** None. **T. Mano:** None. **S. Murayama:** None. **A. Iwata:** None. **T. Toda:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.03/D6

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: Hiv glycoprotein gp120 induces tau hyperphosphorylation via cGMP-dependent kinase II

Authors: ***M. SATHLER**[1], K. SZTUKOWSKI[2], C. MILLER[3], J. H. ELDER[4], S. VANDEWOUDE[3], S. KIM[1];
[1]Biomed. Sci., [2]Col. of Vet. Med. and Biomed. Sci., [3]Microbiology, Immunol. and Pathology, Colorado State Univ., Fort Collins, CO; [4]Immunol. and Mol. Biol., The Scripps Res. Inst., La Jolla, CA

Abstract: Over half of human immunodeficiency virus (HIV)-infected individuals suffer from HIV-associated neurocognitive disorders (HAND), including patients on the combination antiretroviral therapy (cART). As a result of more HIV-infected individuals surviving to older ages due to the efficacy of the treatment, they are at increased risk of developing neurodegenerative disorders. Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder characterized by two anatomical hallmarks, extracellular and intracellular protein aggregates: senile plaques and neurofibrillary tangles (NFTs), composed of beta-amyloid protein (Aβ) and hyperphosphorylated tau, respectively. Although recent studies indicate similar neuropathology between AD and HAND, little is known about mechanisms underpinning neurodegeneration in individuals with HIV.
We quantify neuronal activity by monitoring Ca^{2+} dynamics using intracellular Ca^{2+} imaging after transfection with GCaMP, a genetically encoded calcium sensor in cultured hippocampal neurons. HIV-induced tau phosphorylation (Ser202/Thr205) was measured by immunoblots of cultured cortical neurons. We also employ the Feline Immunodeficiency Virus (FIV) as a model to elucidate the molecular pathways underlying HIV-induced neuronal dysfunction, since it shares its structure, cell tropism, and pathology with HIV, including wide-ranging neurological deficits. Moreover, aged cats develop both amyloid deposits and tau pathology naturally, similar to humans; a feature lacking in all other animal models.
We reveal that HIV and FIV envelope glycoproteins, gp120 and gp95, respectively, interact with the chemokine receptors and facilitates the release of intracellular Ca^{2+}. Significantly, gp120 and gp95 effects on calcium activity are dependent of the cGMP-dependent protein kinase II (cGKII) pathway, which increases Ca2+ release and synaptic activity. gp120 and gp95 were also able to induce tau protein hyperphosphorylation, which is dependent on the activation of p38-MAP

kinase and cGKII, since addition of a p38 blocker (SB203580 10μM) or a cGKII inhibitor (KT5823 1μM) prevented both gp120 and gp95 effects on tau phosphorylation. Moreover, we reveal that human Aβ-specific immunoreactivity (using the 6E10 antibody) are higher in the hippocampal CA1 area of 2-year-old cat brains at 350 days post-infection of FIV. These results thus provide a novel neurobiological mechanism of cGKII-mediated synaptic hyperexcitation in HAND, leading to tau hyperphosphorylation.

Disclosures: **M. Sathler:** None. **K. Sztukowski:** None. **C. Miller:** None. **J.H. Elder:** None. **S. Vandewoude:** None. **S. Kim:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.04/D7

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: NINDS grant (NS102730)

Title: Neuronal hyperactivity and DNA damage in tau P301S mouse model

Authors: *L. LIU, P.-C. PAO, L. A. WATSON, L.-H. TSAI;
MIT, Cambridge, MA

Abstract: Neuronal hyperactivity and DNA damage have been increasingly linked to cognitive decline in aging brains and neurodegenerative disorders. We have previously revealed that DNA double-strand breaks (DSBs) precede the appearance of neurological symptoms in Tau P301S mouse model of tauopathy at 2 months of age. In addition, we have also demonstrated that neuronal activity results in the formation of DNA DSBs. However, whether elevated levels of DSB are caused by neuronal hyperactivity in 2-month-old Tau P301S mice remains elusive. Here, we assess the electrophysiological properties of hippocampal pyramidal neurons in Tau P301S mice and non-transgenic littermates as control at 2 months of age. We observe elevated DNA damage along with increases in both frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) in hippocampal pyramidal neurons of Tau P301S mice. Moreover, hippocampal pyramidal neurons in Tau P301S mice exhibit aberrant hyperexcitability. Together, these findings indicate that hippocampal pyramidal neurons from Tau P301S mice exhibit neuronal hyperactivity and hyperexcitability, concomitant with increased DNA damage in the brain.

Disclosures: **L. Liu:** None. **P. Pao:** None. **L.A. Watson:** None. **L. Tsai:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.05/D8

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: Development of an *in silico* model of tau progression based on *in vivo* measurements of selected relevant parameters

Authors: *J.-P. SOUCY[1], F. MOHAMMADI[2], V. HORTELAN[2], T. BOULIER[3], P. ROSA-NETO[4], T. A. PASCOAL[5], M. SAVARD[4], M. KANG[4], S. MATHOTAARACHCHI[4], J. THERRIAULT[6], H. BENALI[1];
[1]PERFORM Ctr., [2]Concordia Univ., Montreal, QC, Canada; [3]Sorbonne Universities, Paris, France; [4]McGill Ctr. for Studies in Aging, McGill Univ., Montreal, QC, Canada; [5]McGill Ctr. for Studies in Aging, McGil Univ., Montreal, QC, Canada; [6]Psychology, McGill, Montreal, QC, Canada

Abstract: In Alzheimer's disease (AD), misfolded/aggregated amyloid β (Aβ) and tau show evidence of prion-like behavior with different "strains" showing varying tissue distribution/virulence. Disrupting their spreading might modify disease course and is currently being tested. Many steps of the spreading processes can be targeted, preferentially critical ones, but defining those is difficult. We are therefore developing, for tau, an *in silico* model of spreading which could be used for that purpose. Tau concentration in a brain region is constrained by a complex system of biochemical, physical and anatomical parameters, including: a) production/degradation rates of peptide aggregates, likely variable across neuronal/glial cells types, and decreasing as cell losses mount; b) formation of different strains due to variant amino acids sequences/length and evolving local biochemical conditions; c) their physical dispersion by intra/extra-cellular diffusion, axonal transport and synaptic release and uptake (also affected by progressing gray/white matters losses); d) modulation of toxicity by co-localized toxic species (Aβ for instance). All of those are influenced by genetic, epigenetic and environmental factors. A model including all of the above is likely intractable, but we hope to identify those with the most impact on *in vivo* tau distribution. Our initial approach combined Wegner & Engel's (1975) description of tau aggregation kinetics, subjects-specific measured (PET) tau local abundance (SUVR) and a model of spreading mechanisms for tau species (heat/mass transport equations, water diffusion model from subjects' MRI studies). Model results over operator-specified time frames were compared to individuals', not averaged projected, progression in 34 Alzheimer's subjects (ADNI) with initial T1 and DWI MR studies and 2 PET tau studies. Based on those inputs only, we found no mean SUVR progression between PET1/2 in the DMN (p: 0.97) due to a counterbalancing mix (17/17) of subjects showing increasing or decreasing tau concentrations. Absence of regional increase was not initially envisioned in the model and therefore,

unsurprisingly, simulations and PET 2, even when limited to "*global* progressors", differed significantly (p: 0.003). We are showing here that non progression is associated with more initial atrophy in regions where this happens, likely indicating more advanced disease with declining production/transportation of tau, as well as in areas connected to those. We are now working on including atrophy in our model. We also are currently evaluating the impact of regional Aβ SUVRs as an independent determinant of tau distribution progression.

Disclosures: **J. Soucy:** None. **F. Mohammadi:** None. **V. Hortelan:** None. **T. Boulier:** None. **P. Rosa-Neto:** None. **T.A. Pascoal:** None. **M. Savard:** None. **M. Kang:** None. **S. Mathotaarachchi:** None. **J. Therriault:** None. **H. Benali:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.06/D9

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: NSF GRFP DGE-1650116
NIH NIA Grant 1R21AG058080-01
Alzheimer's Association Grant NIRG-14-321219
AFAR Grant RAG15247
CurePSP

Title: The role of deacetylation in tau mediated neurodegeneration

Authors: *H. TRZECIAKIEWICZ[1], D. AJIT[1], J.-H. TSENG[1], Y. CHEN[1], S. MOY[1], D. IRWIN[2], T. COHEN[1];
[1]Univ. of North Carolina, Chapel Hill, NC; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Tauopathies are a group of progressive neurodegenerative diseases marked by the accumulation of aberrantly modified tau proteins. In particular, acetylated tau has been recently implicated in neurodegeneration and cognitive decline. Interestingly enough, acetylation abundantly decorates the microtubule (MT) binding region, thereby inducing the pathophysiological conversion of MT binding tau to aggregated tau. However, with the recent emergence of tau acetylation, it remains unclear which acetyltransferases and deacetylases regulate neuronal tau acetylation. While sirtuins and histone deacetylases (HDACs) are implicated in aging and neurodegeneration, we show that HDAC6 in particular mediates tau deacetylation. Therefore, we aimed to explore the role of HDAC6 in tau pathogenesis and disease progression. Here, we discuss a novel mechanism explaining how HDAC6 modulates aberrantly modified tau proteins. Furthermore, using human Alzheimer's disease (AD) brains, we determined a correlation with disease progression. Importantly, we evaluated the impact of

manipulating HDAC6 *in vivo*, by generating a new tauopathy mouse model followed by pathology, cognition, and survival analysis. We discuss the implications of HDAC6 as a critical regulator for the status of tau acetylation, tau pathogenesis, and cognitive function. Our results shed light on the therapeutic utility of targeting HDAC6 in AD and other related tauopathies.

Disclosures: H. Trzeciakiewicz: None. **D. Ajit:** None. **J. Tseng:** None. **Y. Chen:** None. **S. Moy:** None. **D. Irwin:** None. **T. Cohen:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.07/D10

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: BBSRC funded doctoral fellowship
ARUK small project grant

Title: Introduction of tau oligomers into cortical neurons alters action potential dynamics and disrupts synaptic transmission and plasticity

Authors: *E. HILL[1], T. K. KARIKARI[3], K. G. MOFFAT[1], M. J. E. RICHARDSON[2], M. J. WALL[1];
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Abstract: Tau is a highly soluble microtubule-associated protein that acts within neurons to modify the stability of microtubules. Abnormally phosphorylated tau can dissociate from microtubules and form oligomers and fibrils which associate in the soma-dendritic compartment. Although tau can form neurofibrillary tangles (NFTs), whose abundance correlates with the severity of tauopathies such as Alzheimer's disease (AD), it is the soluble oligomers that appear to be the toxic species. There is, however, relatively little quantitative information on the concentration- and time-dependent actions of soluble tau oligomers (oTau) on the electrophysiological and synaptic properties of neurons. Here, *in vitro* whole-cell patch clamp recording was used to introduce, via the patch pipette, known concentrations of recombinant oligomeric full-length tau-441 into mouse hippocampal CA1 pyramidal and neocortical layer-V thick-tufted pyramidal cells. This allowed the measurement of changes in electrophysiological parameters, basal synaptic transmission and synaptic plasticity. This approach also enabled the evaluation of the direct effects of oTau within a neural network that was otherwise free from tau pathology. In single CA1 cells, oTau significantly increased input resistance, reduced action potential amplitude and slowed action potential rise and decay kinetics. These results were not

observed following the introduction of either vehicle or monomeric Tau. Re-patching experiments confirmed the observations were not due to aggregating oTau occluding the pipette tip. Using our targeted approach, oTau was introduced into either pre- or postsynaptic cells and the effects on synaptic transmission and plasticity measured. oTau introduced into presynaptic neurons induced the run-down of unitary EPSPs which was associated with increased short-term depression. In contrast, introduction of oTau into postsynaptic neurons had no effect on basal synaptic transmission, but markedly impaired the induction of long-term potentiation in the hippocampus. The project will now focus on understanding the mechanisms underlying these observations. We have established a proof of principle in the successful performance of these studies that in the future will be extended for in-depth analysis of different oTau preparations and for tau extracts sourced from the brains of AD patients.

Disclosures: E. Hill: None. **T.K. Karikari:** None. **K.G. Moffat:** None. **M.J.E. Richardson:** None. **M.J. Wall:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.08/D11

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: NIH R56 AG057469

Title: Dysregulation of exosome cargo by mutant tau expressed in human-induced pluripotent stem cell (iPSC) neurons, revealed by proteomics analyses

Authors: *S. PODVIN[1], Q. LIU[1], A. JONES[1], C. MOSIER[1], C. LIETZ[1], L. RANSOM[1], T. IKEZU[2], R. RISSMAN[1], S. YUAN[1], V. HOOK[1];
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Abstract: Accumulation of tau as neurofibrillary tangles (NFT) participates in the neuropathology of human Alzheimer's disease (AD) and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Multiple tau gene mutations are pathogenic, resulting in tau aggregation. Mutant tau RD-LM expression, containing the P301L and V337M mutations, results in NFT formation in human-induced pluripotent stem cell (iPSC) neurons (Reilly et al., 2017). These tau RD-LM expressing human neural cells release tau-containing exosomes, extracellular vesicles. Injection of these tau exosomes into mouse brain results in transfer and accumulation of human tau into neurons (Reilly et al., 2017), and spreading of tau pathology in brain (Winston et al., 2019). These findings suggest the hypothesis the presence of unique cargo in tau-RD-LM exosomes compared to those from control iPSC neurons. To test this

hypothesis, we conducted global proteomics analyses of the exosomes generated from tau RD-LM expressing iPSC neurons and control iPSC neurons. Notably, data illustrated significant differences in the proteome cargo of mutant tau exosomes compared to controls. Specifically, proteomics data identified 606 proteins in the two groups of exosomes. Proteins were categorized as (a) present only in mutant tau exosomes (19 proteins), (b) present only in control exosomes (246 proteins), and (c) present in both mutant tau and control exosomes (352 proteins) with quantitative comparisons for up- and down-regulated components in the tau compared to control exosomes. Gene ontology (GO) and STRING analysis was conducted to gain knowledge of functional protein systems represented by the identified exosome proteins. Proteins found only in tau-RD-LM exosomes were related to clathrin mediated endocytosis. Proteins found only in control exosomes were associated with cell localization and compartmentalization. Significantly down-regulated proteins in tau exosomes represent functions of regulated exocytosis, secretory vesicles, myelin sheath, and related. Significantly up-regulated proteins in tau versus control exosomes represent functions of cellular component organization and cellular components of secretory granules, extracellular matrix, and related. Western blots showed elevation of total tau in mutant tau exosomes compared to controls. These exosomes also contain phospho-tau (Ser396), known to be present in neurally-derived exosomes from plasma of AD patients (Winston et al., 2016). In summary, these findings demonstrate that mutant tau RD-LM dysregulates the exosome proteome cargo which participates in accumulation and spreading of tau in mouse brain.

Disclosures: S. Podvin: None. **Q. Liu:** None. **A. Jones:** None. **C. Mosier:** None. **C. Lietz:** None. **L. Ransom:** None. **T. Ikezu:** None. **R. Rissman:** None. **S. Yuan:** None. **V. Hook:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.09/D12

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: The diverse activities of TTBK isoforms are regulated by extracatalytic regions and autophosphorylation

Authors: *C. BAO, G. DILLON, B. BAJRAMI, H. CERTO, S. KOIRALA, D. RABAH; Biogen, Cambridge, MA

Abstract: Tau tubulin kinase 1 (TTBK1) is a CNS-specific serine/threonine and tyrosine kinase that has been implicated in the pathological phosphorylation of tau in Alzheimer's Disease (AD) and Frontotemporal Dementia (FTD). TTBK1 as a therapeutic target may prove challenging because it shares a highly conserved catalytic domain with its homolog, TTBK2, a ubiquitously-expressed protein that is genetically linked to spinocerebellar ataxia type 11. This kinase

homology poses an important consideration when designing selective compounds for TTBK1 and avoiding the potential off-target effects of inhibiting TTBK2. Elucidating the distinctions and similarities between TTBK1 and TTBK2 will increase our understanding of them as distinct targets in the treatment of neurodegenerative disease. To investigate whether isoform-specific substrates and/or interacting partners could be involved in the divergent roles of these kinases in disease, we examined the consequences of manipulating TTBK1/2 expression across several cellular models. In HEK293T cells we demonstrate that TTBK1 has a significantly greater affinity for phosphorylating tau at disease relevant sites when compared to TTBK2. Importantly, we also demonstrate in primary mouse neurons that knocking down endogenous TTBK1, but not TTBK2, can significantly attenuate the tau phosphorylation induced by okadaic acid treatment. Together these results implicate TTBK1 as the isoform responsible for tau phosphorylation in the brain. To determine how the unique kinase activities of each TTBK isoform are regulated, we made several truncation constructs of TTBK1 and TTBK2. Our data indicates that the truncated kinase domains of TTBK1 and TTBK2 do not exhibit phosphorylation activity in the absence of aa297-770(TTBK1) or aa 346-586(TTBK2), indicating that differences in substrate phosphorylation could be governed by specific amino acids sequences that lie outside of the kinase domain. In addition, we show that both TTBK1 and TTBK2 are heavily autophosphorylated under basal conditions and identify novel TTBK1 autophosphorylation sites *in vivo* that regulate its kinase activity. Finally, we have identified unique interactors for TTBK1 and TTBK2 in rat cortical neurons using proximity-labeling methodology BioID2 coupled with tandem mass spectrometry. Our findings suggest that both the extracatalytic structures and autophosphorylation of TTBK isoforms are responsible for regulating their divergent kinase domain activities. This addresses an important gap in knowledge regarding the implications of targeting TTBK1/2 and may prove valuable in the development of potential therapies for AD and FTD.

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Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.10/D13

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: FRQS Fellowship 32289 and 36470
BU ADC - NIH/NIA P30-AG013846
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NIA/NINDS R01AG057902

Title: Pathological correlates of depressive and suicidal phenotypes across brain regions in chronic traumatic encephalopathy

Authors: *I. MAHAR[1], B. R. HUBER[1,2], T. D. STEIN[1,2], V. E. ALVAREZ[1,2], A. C. MCKEE[1,2];
[1]AD & CTE Ctr., Boston Univ., Boston, MA; [2]Boston VA Med. Ctr., Boston, MA

Abstract: BACKGROUND
Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease with cognitive, behavioral, and psychiatric symptoms, with depression and suicidality present in a large proportion of pathologically validated CTE cases to date. Pathologically CTE is defined by a distinctive accumulation of hyperphophorylated tau (pTau) predominately involving the frontal and temporal cortices, medial temporal lobe and brainstem. However, the association between pathology in particular brain regions and psychiatric phenotypes in CTE is currently unknown.
METHODS AND RESULTS
Human postmortem brains from CTE cases and controls were obtained from the Veterans Affairs - Boston University - Concussion Legacy Foundation (VA-BU-CLF) Brain Bank (N=320). A team of trained neuropathologists examined each brain (blinded to identity) for gross and microscopic pathology, and provided semi-quantitative assessments. Brain regions of interest were isolated and sectioned as reported previously (Mez et al. 2015 Alzheimers Res Ther 7:62), including frontal, temporal, limbic, and brainstem structures. Sections were stained for pathological markers of interest (e.g. pTau, β-amyloid, α-synuclein, pTDP43, etc). Stained slides were scanned and traced digitally, with staining quantified using a Leica Aperio system. Preliminary analyses suggest that alterations in specific limbic, midbrain, and white matter structures may associate with depressive and suicidal phenotypes in CTE. In particular, white matter loss and hippocampal and nigral pTau pathology were increased in depressed cases. We have also found inflammation-associated changes in the anterior cingulate cortex of depressed CTE cases.
CONCLUSIONS
This ongoing investigation is the first to explore the association between neuropathology in particular brain regions and specific psychiatric phenotypes in CTE. Preliminary results suggest that increased neurodegenerative pathology, loss of white matter, and neuroinflammation could underlie depressive symptoms in CTE. We are currently adding analyses and markers in order to validate and extend our initial analyses and findings.

Disclosures: I. Mahar: None. **B.R. Huber:** None. **T.D. Stein:** None. **V.E. Alvarez:** None. **A.C. McKee:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.11/D14

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: The master circadian clock protein BMAL1 strongly modulates neuropathology in a mouse tauopathy model

Authors: *P. W. SHEEHAN, C. J. NADARAJAH, J. DIMITRY, E. S. MUSIEK; Washington Univ. In St. Louis, St. Louis, MO

Abstract: Circadian rhythm dysfunction is a well-known symptom of many neurodegenerative diseases, but recent evidence has also implicated impaired circadian clock function in disease pathogenesis. Our lab has demonstrated that global post-natal deletion of the core circadian clock gene *Bmal1*, which disrupts all circadian function, causes gliosis and oxidative stress in the mouse brain, and increases amyloid plaque pathology in mouse model of Alzheimer's disease (AD). Aggregation of the microtubule-associated protein tau is a critical pathogenic event in AD and many other neurodegenerative tauopathies. We hypothesized that *Bmal1* deletion would exacerbate tau aggregation and downstream pathology in mice. To test this, we generated tamoxifen-inducible global *Bmal1* knockout mice expressing the human *MAPT* transgene bearing the P301S mutation (CAG-Cre^{ERT2};Bmal1(f/f);P301S mice, termed BMKO-T), which were behaviorally arrhythmic under constant conditions after tamoxifen treatment at 2 months of age. At 8 months old, male BMKO-T mice accumulated similar amounts of AT8+ hyperphosphorylated tau as Cre- control P301S mice. However, BMKO-T mice exhibited significantly less insoluble and misfolded tau than P301S mice, suggesting that *Bmal1* deletion mitigates tau aggregation. BMKO-T mice also showed strikingly diminished microglial activation and decreased expression of damage-associated microglial transcripts when compared to Cre- control P301S mice. Preliminary evidence also suggests a preservation of synapses and hippocampal volume in BMKO-T mice. Our findings suggest that Bmal1 deletion exerts disparate effects on extracellular Aβ and intracellular tau aggregation, and suggest that Bmal1 deletion protects the brain from tau aggregation, perhaps via activation of glia.

Disclosures: P.W. Sheehan: None. **C.J. Nadarajah:** None. **J. Dimitry:** None. **E.S. Musiek:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.12/D15

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: AG016976
AG43511
Cure Alzheimer's Fund

Title: Tau seeding activity closely correlates with number of tangles and synaptic phospho-Tau in brain regions corresponding to different Braak stages

Authors: *P. RAMANAN, A. AMARAL, M. MARQUIE, A. ANTON-FERNANDEZ, N. SAEZ-CALVERAS, M. SIAO TICK CHONG, C. AGUERO, T. GOMEZ-ISLA;
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Abstract: BACKGROUND: The development and spreading of tau pathology in Alzheimer's disease (AD) follows a stereotypical spatiotemporal pattern of progression from entorhinal, to limbic, to the isocortical areas as described by Braak and Braak. It has been shown that AD brain extracts can seed tau aggregation in cell cultures expressing fluorescently tagged tau constructs, favoring the idea that tau seeds can propagate through the brain via cell to cell transfer. Recent studies in human brains at different Braak stages have reported widespread tau seeding activity in regions predicted to be free of tau deposits suggesting that transcellular propagation of tau seeds precedes subsequent development of pathology. However, the relationship between tau seeding activity and detailed quantitative measures of neurofibrillary tangles (NFT) and soluble phospho-Tau (p-Tau) species in the regions corresponding to the different Braak stages has not been investigated in depth. GOAL: To study the correlation of tau seeding activity with number of NFT and content of soluble p-Tau in synapses in AD brains representing the spectrum of Braak staging (from Braak I-VI). METHODS: We performed stereological counts of NFT and measured levels of soluble p-Tau in synaptoneurosome preparations by Western Blot and ELISA in postmortem tissue containing entorhinal cortex (EC), superior temporal sulcus (STS) and visual cortex (VC) from 22 brains representing the different Braak stages: low Braak (I-II, n=6), intermediate Braak (III-IV, n=7), and high Braak (V-VI, n=9). We analyzed the relationship of those measures with tau seeding activity, as reported by HEK TauRDP301S-CFP/YFP cell assay (Holmes et al. 2014), in the same regions of interest, using PBS-soluble lysates. RESULTS: Tau seeding activity positively correlated with Braak staging, with high Braak cases showing higher seeding activity than low Braak cases across all regions. Within each Braak stage, the highest tau seeding activity was observed in the EC, followed by the STS, and then VC. Across the spectrum of cases, tau seeding activity was significantly correlated with number of NFT and levels of

synaptic p-Tau. Tau seeding activity was negligible in regions free of NFT or synaptic p-Tau. CONCLUSION: Our data suggest that tau seeding activity in the human AD brain, as measured by HEK-tau-biosensor cell assay, closely correlates with other measures of tau pathology (NFT and synaptic p-Tau) in brain regions corresponding to the different Braak stages. These data could be relevant to inform current efforts aimed at ultrasensitive tau-seeding activity assays as a potential biomarker in AD.

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Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.13/D16

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: NIH Grant AG050431
Alzheimer's Association Zenith Fellows Award ZEN-17-438829

Title: Activation of microglia by tau fibrils

Authors: *M. MAJUMDER[1], D. DUTTA[2], K. PAHAN[3];
[1]Dept. of Neurolog. Sc, [2]Dept. of Neurolog. Sci., [3]Dept Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Alzheimer's disease (AD) is the most common form of dementia and microglial activation plays an important role in the pathogenesis of different neurodegenerative disorders including AD. Despite intense investigations, no effective therapy is available to stop the progression of AD. Since tangles containing hyperphosphorylated tau are important pathological features of AD, here we examined the role of fibrillar tau in microglial activation. Fibrillar tau increased microglial activation as evidenced by increased mRNA expression of inducible nitric oxide synthase (iNOS) and interleukin-1β (IL-1β). Microglial activation was also supported by immunofluorescence analysis. Since NF-κB is an important proinflammatory transcription factor, we examined the activation of NF-κB by fibrillar tau. Induction of NF-κB-driven luciferase reporter activity and increase in p65 phosphorylation in fibrillar tau-stimulated microglia suggested activation of NF-κB by fibrillar tau. It has been shown that peptides corresponding to the NF-κB essential modifier (NEMO)-binding domain (NBD) of IκB kinase α (IKKα) or IκB kinase β (IKKβ) specifically inhibit the induction of NF-κB activation without inhibiting the basal NF-κB activity. Accordingly, here, we also describe that wtNBD peptide inhibited fibrillar tau-induced expression of proinflammatory molecules in microglia. These

results suggest that tau fibrils activate microglia via NF-κB and that NBD peptide may have therapeutic importance in AD.

Disclosures: **M. Majumder:** None. **D. Dutta:** None. **K. Pahan:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.14/D17

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: Rab35 in tauopathy disorders

Authors: *M. S. PARMAR[1], S. A. KOREN[2], G. BAE[1], M. R. FILORAMO[1], J. F. ABISAMBRA[2], N. R. MCFARLAND[1];
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Abstract: Rab GTPase proteins play an important role in vesicular trafficking, endocytosis, and endosomal-lysosomal pathways, and are increasingly implicated in neurodegenerative disease pathology such as Alzheimer's (AD). In AD the normal function of specific Rab proteins is disrupted early and may contribute to abnormal tau accumulation and neurofibrillary tangle formation. Among several Rab GTPases, Rab35 has an emerging role in Parkinson disease (PD) as potential biomarker. Rab35 levels in PD are increased in serum and overexpression of Rab35 has been shown to exacerbate α-synuclein aggregation and dopamine cell toxicity in both cell and animal models. In contrast, tau pathology in a rat model induced by glucocorticoid-mediated stress is associated with reduction in Rab35. Overexpression of Rab35 was able to rescue these changes. Analysis of a proteomics dataset from quantitative TMT-labelled human brain extracts derived from AD further demonstrates a significant reduction of Rab35 levels in diseased affected regions, such as the frontal cortex and anterior cingulate gyrus. Together, these data suggest that Rab35 may regulate tau accumulation and associated pathology. In this study we examined the expression levels of Rab35 protein in postmortem human brain samples from multiple tauopathies, such as AD, Corticobasal Degeneration (CBD) and Progressive Supranuclear Palsy (PSP) and matched healthy controls. Tissues from select brain regions including frontal and temporal cortex, striatum, and white matter were analyzed. Frozen brain samples were homogenized in the high salt buffer and analyzed by Western blot. Rab35 protein expression was significantly decreased in the striatum tissues of all tauopathies and in the CBD white matter tissue. Rab35 protein expression was also lower in the frontal cortex tissues. To test the hypothesis that overexpression of Rab35 can rescue human tau accumulation and aggregation, human neuroglioma H4 cells were co-transfected with 4R0N WT or self-aggregating 2X Tau (double mutant P301L/S320F) with WT Rab35 and dominant negative (DN)

Rab35 S22N. Rab35 overexpression resulted in a marked reduction of both total and phospho - soluble and insoluble Tau. In addition, Rab35 overexpression resulted in a reduction in ThioS positive tau inclusions *in vitro*. These data provide the first evidence that Rab35 may play an important role in human tauopathy disorders. The findings from these studies provide preliminary data for larger studies examining the impact of Rab proteins in tauopathy and their potential for therapeutic modulation.

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Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.15/D18

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: Directs current stimulation reduces tau-induced neurodegeneration

Authors: *V. MOROZOVA, A. GORIN, Z. AHMED, A. ALONSO;
Col. of Staten Island, Staten Island, NY

Abstract: Pathological human tau (PH-Tau) is a pseudophosphorylated form of tau protein that mimics the effects of tau isolated from Alzheimer's disease patients. Recently we found that when PH-Tau is added to a cell media of cultured neurons it can be taking up by these neurons, resulting in the accumulation of proteins in the somatodendritic compartment as well as the disruption of microtubules and neurodegeneration (Morozova et al., unpublished). Also, we demonstrated that Direct current (DC) stimulation has an effect on stem cell migration, proliferation and differentiation (Samaddar et al., 2017). Besides that, we showed that DC Stimulation promotes the upregulation of heat shock proteins (Mekhael et al., 2019), which are responsible for the identification and degradation of misfolded proteins. Meanwhile, the experiments done to investigate the effects of DC stimulation at the cellular level are very scarce, there are even less studies that seek to explore whether DC stimulation can be used to reduce/slowdown neurodegenerative conditions similar to those seen in Alzheimer's disease. This study was designed to investigate the effects of DC stimulation on healthy neurons and neurons undergoing neurodegeneration. We hypothesize, that an increase in heat shock proteins due to DC stimulation would result in the enhancement of misfolded protein degradation which

may slow down neurodegeneration in neurons exposed to PH-Tau. The addition of PH-Tau to the primary neuronal cultures was used to determine if DC stimulation will lead to reduction of cytoplasmic PH-Tau and slow down neurodegenerative processes initiated by the protein. Our results demonstrated that stimulated neurons had reduced PH-Tau accumulation compared to unstimulated cultures. Our data showed that DC stimulation did not only prevented neurodegeneration caused by PH-Tau but also enhanced cell growth and resulted in changes in morphology. Also, we found increase in HSP70 levels in stimulated cultures. In cultures that were pre-treated with TNFα or MG-132 (degradation blockers), accumulation of PH-Tau was similar to unstimulated cultures, indicating that reduction of PH-Tau in stimulated cultures may be due to the upregulation of a protein degradation pathway. Currently, we are studying the effects of DC stimulation on neurodegeneration *in vivo* using our transgenic mouse model with conditional PH-Tau expression. We propose that DC stimulation will prevent tau-induced neurodegeneration via upregulating degradation pathways.

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Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.16/D19

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: NIH R01NS077284

Title: PERK-tau complex promotes pathogenic outcomes in tauopathy models

Authors: *D. A. GILLETT[1], S. KOREN[2], S. E. MEIER[4], J. F. ABISAMBRA[3];
[2]Ctr. for Translational Res. in Neurodegenerative Dis., [3]Neurosci. & Ctr. for Translational Res. in Neurodegenerative Dis., [1]Univ. of Florida, Gainesville, FL; [4]Univ. of Kentucky - Sanders Brown Ctr. On A, Lexington, KY

Abstract: A major challenge in the field of neurodegeneration is the identification and successful manipulation of therapeutic targets to improve clinical outcomes. Multiple studies have focused on PERK and tau. Increased levels or accumulation of pathogenic forms of these proteins correlate with neurodegeneration in tauopathies, yet conflicting results have failed to coalesce into a common mechanism. Our recent data using cell-free, cell culture, and *in vivo* models suggest that PERK and tau form a stable complex, that this interaction favors tau phosphorylation, and that this phenomenon decreases translation; this latter effect is feature of tauopathic processes. In order to further characterize this interaction, we co-immunoprecipitated tau mutants associated with pathology and determined mutant-specific effects that affect the interaction. We then determined the degree to which PERK activity modifies tau dynamics.

These data highlight for the first time a direct PERK-tau interaction that drives pathogenic outcomes. As such, we identify a potentially targetable mechanism to alleviate the devastating consequences of tauopathy.

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Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.17/D20

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: NIH RO1 F041854
NIH T32-NS007222-36UM
NIH F32 AG059362-01

Title: UBQLN2 alters tau aggregation dynamics *in vivo*

Authors: J. E. GERSON[1], J. WELDAY[2], J. D. GREGORY[2], H. L. PAULSON[3];
[1]Neurol., [2]Univ. of Michigan, Ann Arbor, MI; [3]Prof, Neurol, Univ. of Michigan Dept. of Neurol., Ann Arbor, MI

Abstract: UBQLN2 has emerged as a protein quality control factor that likely contributes to numerous neurodegenerative diseases characterized by protein aggregation. UBQLN2 is a member of a family of brain-expressed ubiquilin proteins that possess ubiquitin-like and ubiquitin-associated domains that enable shuttling of ubiquitinated proteins to the proteasome for degradation. UBQLN2 missense mutations cause frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) and UBQLN2 colocalizes with aggregates in Huntington's disease and FTD/ALS. Knowledge of the interaction between UBQLN2 and tau pathology, however, is limited, particularly under normal conditions or earlier disease stages. To evaluate whether UBQLN2 regulates tau clearance, we assessed levels of tau in human embryonic kidney-293 cells with and without UBQLN2. Co-expressed UBQLN2 markedly lowered levels of tau. Conversely, siRNA knockdown of UBQLN2 significantly elevated levels of tau. To determine whether UBQLN2 acts on tau in a similar manner in the central nervous system *in vivo,* tau transgenic mice were crossed with UBQLN2 transgenic and *Ubqln2* knockout mice. Surprisingly, UBQLN2 did not broadly decrease total tau levels in the brain as it did in a cellular model. However, UBQLN2 altered specific aggregated and post-translationally modified species

of tau, increasing phosphorylated tau and insoluble poly-ubiquitinated substrates, suggesting a dynamic relationship between tau and UBQLN2 that warrants further study. The possibility that UBQLN2 also undergoes alterations in disease was evidenced by the fact that increased insoluble, high molecular weight UBQLN2 was detected in both brains from transgenic mouse models of tauopathy and human progressive supranuclear palsy mid-frontal gyrus when compared to controls. Our findings highlight a new role for UBQLN2 in managing tau and suggest that changes to UBQLN2 in disease could alter the dynamics of proteostasis, affecting tau toxicity. A better understanding of the impact of UBQLN2 and other components of protein clearance pathways is needed to fully elucidate how alterations in proteostasis occur in disease and affect toxicity associated with tau pathology.

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Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.18/D21

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: Isoform-specific modulation of spines and neuronal morphology by tau

Authors: M. RIEROLA, R. BRANDT, *L. BAKOTA;
Univ. of Osnabrück, Osnabrück, Germany

Abstract: Tau is a microtubule-associated protein known to play a determinant role in several neurodegenerative diseases, designated as tauopathies. Tau´s interaction with microtubules is well studied however, it is unclear how different isoforms of tau, such as fetal (352aa) or the longest adult (441aa) CNS tau might influence neuronal connectivity as evidenced by changes in dendritic spines and neuronal morphology. Here we address this question by using an *ex vivo* model of hippocampal slice cultures from tau knockout (tau KO) and control C57BL/6 (B6) seven days-old mice. Cultured hippocampal tissues were exposed to a viral vector expressing either human fetal or adult tau isoforms fused with a fluorescent protein. In addition, the contribution of exon 1 was analyzed using a respective deletion construct. 3D confocal images were taken from: i) dendritic segments of CA1 pyramidal neurons, in order to study dendritic spine parameters (n=31-40 segments); and ii) the whole apical arbor (n=7-10 neurons) to determine if tau influences neuronal morphology. Images were blind deconvolved and further analyzed by algorithm-based methods. Our results reveal that the presence of fetal or adult human tau on tau KO background leads to a decrease in spine density compared to control slices of the same genotype or slices from B6 mice. Deletion of exon 1 (ΔE1) from the adult tau isoform leads to further decrease in spine density. Neurons expressing ΔE1 construct also show a significantly reduced length of the apical branches. Surprisingly however, the analysis of the

fraction of mature spines shows that the presence of adult tau leads to an increase in the fraction of mushroom spines (with or without exon 1), while fetal tau compromises the fraction of this spine type compared to control samples. Since tau's exon 1 has been demonstrated to interact with several plasma membrane associated proteins, such as Annexin A2 and Annexin A6, we conclude that its absence is also compromising the post-synaptic compartment. Our data also suggest that fetal tau, which lacks an interacting domain to microtubules due to splicing (3R), affects the maintenance of mature spines compared to adult tau (4R). This finding is consistent with the idea that during development more dynamic spines, such as thin and stubby spines, may be required.

Disclosures: M. Rierola: None. **L. Bakota:** None. **R. Brandt:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.19/D22

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Title: Structural and functional analysis of hippocampal CA1 neurons in mice lacking tau expression

Authors: M. HRYNCHAK, M. RIEROLA, N. ABREU, *R. BRANDT, L. BAKOTA; Neurobiologie der Univ. Osnabrück, Osnabrueck, Germany

Abstract: Microtubules (MTs), the main component of the cell skeleton, provide the track for axonal transport, are required for structural integrity of neurons, and affect neuronal plasticity. Due to their vital functions during neuron development and function, MT destabilization could be directly or indirectly connected to the mechanisms leading to neurodegeneration. Tau, a microtubule-associated protein involved in MT assembly and regulation of MT dynamics, is not only an axon-specific protein but it is also found in the somatodendritic compartments. Due to its clear implication in a class of neurodegenerative diseases attempts are made to lower or diminish the protein tau, as a treatment possibility. Therefore, it is crucial to learn how this would affect the neuronal network function, how it might alter neuronal structure and signalization and whether this is mediated in a MT-dependent or independent manner. In this regard, the morphology and function of CA1 neurons from tau knock out (tauKO - B6.129X1-Mapt^{tm1Hnd}/J) 12 month-old male mice had been studied. Algorithm-based analysis of 3D micrographs of apical dendrites revealed that the spine density remains unchanged between control (thy1-GFP line M) and tauKO mice. However, there are marked differences in fraction of mushroom, thin and stubby spines between the two genotypes. Furthermore, a similar length of apical dendritic tree could be observed after a reconstruction in 3D by semi-automated tracing of the fluorescence signal. Analysis of potential functional alterations in synaptic transmission was

performed by microelectrode array (MEA) system. Induction of LTP either by theta-burst stimulation or high-frequency stimulation (HFS) lead to a higher potentiation of the population spikes in the tauKO mice however, significantly increased LTP response between the two genotypes regarding fEPSP could be obtained only after HFS. In the view of the above, lack of tau in 1-year-old male mice leads to substantial structural and functional alterations. Further research is required to understand if these findings in mouse model can be translated into human brain.

Disclosures: **M. Hrynchak:** None. **M. Rierola:** None. **N. Abreu:** None. **L. Bakota:** None. **R. Brandt:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.20/D23

Topic: C.05. Tauopathies/ Tau-dementias/ and Prion Diseases

Support: NIH Grant F041854

Title: High overexpression of UBQLN2 leads to degeneration of the retina

Authors: *S. S. SANDOVAL-PISTORIUS[1,2], L. M. SHARKEY[1], H. L. PAULSON[1];
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Abstract: Ubiquilin-2 (UBQLN2) is one of four ubiquilin proteins that contain N-terminal ubiquitin-like (UBL) and C-terminal ubiquitin-associated (UBA) domains that allow ubiquitinated substrates to be shuttled to the proteasome for degradation. Ubiquilins may also target proteins for degradation by autophagy, although this is less well-established. The importance of ubiquilins in neurodegenerative disease was underscored by the discovery that mutations in UBQLN2 directly cause frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). UBQLN2 also aggregates *in vitro* and *in vivo*, and pathogenic mutations in UBQLN2 tend to increase its aggregation. Studies have shown that the retina is sensitive to changes in activity of protein quality control pathways. For example, increased levels of autophagy lead to degeneration of the retina, whereas increased levels of proteasomal activity are shown to be protective. Here, we have considered the role of UBQLN2 in retinal integrity. We used three different strains of transgenic mice, one that overexpresses wild-type (WT) UBQLN2 at low levels (Ub2-low), a strain that overexpresses WT UBQLN2 at high levels (Ub2-hi), and a strain that overexpresses a mutant form of UBQLN2 (Ub2-P506T). Retinal structure was evaluated using immunofluorescence at 3 weeks, 8 weeks, 6 months and 12 months of age. At 3 weeks of age, all mice showed retinal structure comparable to non-transgenic controls. Starting at 8 weeks of age, Ub2-hi mice have drastic thinning of the retina, approximately 85% of cells in

the outer nuclear layer are lost, whereas Ub2-low and Ub2-P506T mice maintain normal retinal structure. By 6 months of age, Ub2-P506T mice started to exhibit thinning of the retina, while Ub2-low mice continued to show normal structure of the retina. At 12 months of age, P506T mice have drastic thinning of the retinas, comparable to Ub2-hi mice at 6 months of age, whereas the retinal structure of Ub2-low mice remains largely unaffected. Preliminary results suggest that the photoreceptors, are especially susceptible to high overexpression of WT UBQLN2. These results suggest that increased expression of UBQLN2 is detrimental to cells that are sensitive to changes in activity of protein quality control pathways. The retina may serve as a useful landscape to further investigate the role of UBQLN2 in protein quality control.

Disclosures: S.S. Sandoval-Pistorius: None. **L.M. Sharkey:** None. **H.L. Paulson:** None.

Poster

044. Cellular and Circuit Mechanisms in Tauopathies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 044.21/D24

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

Support: NIH Grant AGO56478
NIH Grant AGO55865
Saban Family Foundation
Maurice Marciano Family Foundation

Title: Retinal tauopathy in mild cognitive impairment and Alzheimer's disease patients mirrors cerebral neurofibrillary tangle burden

Authors: *N. MIRZAEI[1], J. SHEYN[1], Y. KORONYO[1], D.-T. FUCHS[1], D. LEE[2], M.-L. B. SELENICA[3], K. L. BLACK[1], C. A. MILLER[4], M. KORONYO-HAMAOUI[1];
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Abstract: Alzheimer’s disease (AD) may not solely be a brain disorder. The neurosensory retina, a developmental and anatomical extension of the CNS, appears to undergo changes classically associated with AD. This is supported by the identification of several pathological hallmarks of the disease in retinas of patients using both non-invasive live imaging tools and post-mortem tissue analysis. These include the characteristic amyloid β-protein (Aβ) plaques, diminished blood flow, thinning nerve fibers and atrophy. Our team has found a progressive accumulation of Aβ plaques in the post-mortem neurosensory retinas of mild cognitive impairment (MCI) and AD patients, which strongly correlates with brain amyloid plaque pathology. Furthermore, higher levels of retinal hyperphosphorylated tau (pTau) – cerebral

aggregation of which is considered a diagnostic criterion – have been recently reported by other groups. Here, we provide evidence of intraneuronal pTau and neurofibrillary tangles (NFTs) in post-mortem retinal tissue of MCI and AD patients, visualized by immunohistochemical detection of AT100 and AT8 antibodies targeting different disease-associated phosphorylation sites. AT100+ pTau is primarily detected in the nerve fiber layer (NFL), retinal ganglion cells (RGCs) and the inner and outer nuclear layers (INL and ONL, respectively). On the other hand, AT8 produces a strong and specific pattern of staining in the inner and outer plexiform layers (IPL and OPL) and can be detected in the RGCs and NFL. Our results reiterate previous findings of retinal tauopathy in AD while providing novel evidence in MCI patients as well as demonstrating the region-specific presence of differentially-phosphorylated tau in the retina. Importantly, retinal pTau levels appear to mirror brain NFT burden in both MCI and AD patients. We are currently investigating other related post-translational modifications of retinal tau and whether these also correlate with cerebral tauopathy in the same individuals. Since the retina and brain share a common embryonic origin and the former remains an integral part of the latter throughout adulthood, deciphering retinal AD pathology may potentially serve as an accurate and accessible biomarker for reliable disease diagnosis and treatment assessment.

Disclosures: **N. Mirzaei:** None. **J. Sheyn:** None. **Y. Koronyo:** None. **D. Fuchs:** None. **K.L. Black:** None. **M. Koronyo-Hamaoui:** None. **M.B. Selenica:** None. **D. Lee:** None. **C.A. Miller:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.01/D25

Topic: C.06. Neuromuscular Diseases

Support: NIH-NINDS, grant# NS091836.

Title: Fast blue vs. cholera toxin B: Which retrograde tracer is better for spinal motoneurons labeling?

Authors: *H. FARID[1], W. B. GELFORD[2], L. L. GOSS[1], T. L. GARRETT[1], S. M. ELBASIOUNY[1,2];
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Abstract: Retrograde tracers have been used in nerve related studies for decades to provide useful information about neural pathways, neuronal transport and axonal regeneration. Since 1971, tracers have varied with regards to types, method of application, and tracer transport mechanisms. Fast-Blue (FB) and Cholera Toxin B (CTB) are two tracers extensively used in

research, but have yet to be compared. FB is a chemical fluorescent dye that emits a blue fluorescence upon excitation, whereas CTB is the beta-subunit of a bacteria-toxin secreted by the bacterium *Vibrio cholerae*. The two tracers differ in composition and uptake mechanism, in which CTB uses receptor-mediated endocytosis whereas FB uses pinocytosis. The goal of this study was to compare these two tracers to determine which tracer is more effective in labelling mouse spinal motoneurons (MN). Accordingly, FB and CTB of different concentrations (FB: 0.1%, 0.2% and 2%, and CTB: 0.05% and 0.1%) were tested. We intramuscularly injected these tracers into the hindlimbs of mice, which were euthanized 3 or 5 days after injection. MN density, average MN intensity, tracer distribution, neurite length and volume were measured for each tracer concentration. Our results show that 0.2% FB at 3 days had the highest intensity value (i.e., bright MN labeling) and mean neurite length. On the other hand, 0.1% CTB at 5 days had higher MN labelling compared to all other groups. Our results show that the intensity of 2% FB is more stable over time, i.e. fading is less, relative to other groups, whereas 0.2% FB and 0.1% CTB intensities decline more over time. Based off our results, 0.2% FB at 3 days or 0.1% CTB at 5 days are optimal tracer conditions to be used in labelling MN in future studies. Furthermore, researchers can use 0.2% FB to obtain optimal results as opposed to 2% FB, which is the standard concentration used in literature, thus revealing economic benefits for future tracer experiments.

Disclosures: **H. Farid:** None. **W.B. Gelford:** None. **L.L. Goss:** None. **T.L. Garrett:** None. **S.M. Elbasiouny:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.02/D26

Topic: C.06. Neuromuscular Diseases

Support: NIH-NINDS, grant #: NS091836

Title: Contrasting changes in Kv2.1 channel expression level between disease-resistant and disease-vulnerable $SOD1^{G93A}$ motoneurons in ALS

Authors: *J. C. HARRIS[1], C. S. I. DRAPER[2], T. L. GARRETT[2], S. M. ELBASIOUNY[1,2]; [1]Biomedical, Industrial, and Human Factors Engin., [2]Neuroscience, Cell Biology, and Physiol., Wright State Univ., Dayton, OH

Abstract: Kv2.1 channels mediate slow-activating K^+ rectifier current within the membrane of spinal motoneurons (MNs), and they are known to co-localize with cholinergic boutons and muscarinic receptors. Although Kv2.1 channels have been suggested to regulate MN excitability, little research has gone into investigating its potential contribution to MN altered excitability in

Amyotrophic Lateral Sclerosis (ALS). Using the male SOD1-G93A transgenic mouse model of ALS and their wild-type (WT) littermates, we examined Kv2.1 cluster area and density in lumbar MNs. In our experiments, MNs were split into their respective types (slow vs. fast) using SK3 immunoreactivity in order to separate and compare the responses of disease-resistant (i.e., $SK3^{+}$) vs. disease-vulnerable (i.e., $SK3^{-}$) MNs. Four key time points were examined: at postnatal (P) days P10, P30, P90, and end-stage (ES; P120-140). These time points represent key disease stages in the G93A line as P10 is when MN cellular abnormalities are first noted before neurodegeneration occurs; P30 is when disease-vulnerable MNs (i.e., fast type, $SK3^{-}$) begin to die; P90 is when disease-resistant MNs (i.e., slow type, $SK3^{+}$) begin to also die and symptoms emerge; and ES is a late disease stage. Our results show that in disease-resistant ($SK3^{+}$) MNs Kv2.1 cluster area does not change relative to WT until ES when they become significantly downregulated. On the other hand, in disease-vulnerable ($SK3^{-}$) MNs Kv2.1 cluster area is upregulated at P90 before it becomes significantly downregulated at ES, relative to WT. Additionally, no changes in Kv2.1 cluster density were detected in disease-vulnerable or disease-resistant SOD MNs, relative to WT, throughout disease progression. Electrophysiological recordings from WT and SOD MNs in the whole-cord in-vitro spinal cord preparation confirmed the upregulation of Kv2.1 channels at P90 by showing significantly depolarized interspike potential in SOD MNs relative to WT. These results provide important information on how disease-resistant vs. disease-vulnerable MNs regulate their excitability in ALS.

Disclosures: **J.C. Harris:** None. **C.S.I. Draper:** None. **T.L. Garrett:** None. **S.M. Elbasiouny:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.03/D27

Topic: C.06. Neuromuscular Diseases

Support: NIH-NINDS, grant#: NS091836

Title: Cell typing of mouse spinal motoneurons using immunohistochemistry markers

Authors: *T. L. GARRETT[1], C. L. WINTERMUTE[2], M. E. MORAN[1], S. M. ELBASIOUNY[1,3];
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Abstract: Alpha motoneurons (MN) have three different cell types: fast fatigable (FF), fatigue resistant (FR) and slow (S) types. Experimental methods to differentiate between cell types are

important as specific cell types are more susceptible to degeneration in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). In ALS, the large, FF cells are the first to die off in the disease and the S cells are more resistant to the disease and die last. In order to investigate the mechanisms underlying their differential vulnerability in ALS, methods to identify and separate the different MN types need to be developed. Work done by others have established markers such as Osetopontin (OPN, a marker for FR and S cells, Misawa et al 2012) and Matrix Metallopeptidase 9 (MMP9, a marker for FF cells, Kaplan et al 2013) to label S/FR and FF cells, respectively. In the present study, we aimed to expand and refine that methodology in order to label and separate all three MN types successfully. To achieve that, we combined OPN and MMP9 markers with the SK3 channel isoform, a selective marker for S cells (Deardorff et al 2013). In addition to the three cell type markers, MNs were also labeled with VAChT, an alpha MN marker as a secondary confirmatory label. We tested the markers on adult lumbosacral spinal cord tissue from male SOD1 G93A-HC (SOD) mice (n=3) and their wild type (WT, n=3) littermates at postnatal day 90. Lumbar segments L3 through L6 were analyzed for total number of MNs, MN type and MN size. Our results show that although the average size for FF, FR and S cells are different, there is a large overlap in MN size with no clear size cutoffs that separate the three cell types. Our results also show a significant increase in MN size across the lumbar segments in which cell size of L3 MNs was smaller than that of L5 and L6 MNs. In SOD mice, the density of FF and FR MNs was reduced relative to WT, whereas the density of S MNs was unchanged, confirming the known differential effect of ALS on MN types. In sum, our results demonstrate that mouse MNs cannot be typed by their soma size alone and a combination of immunohistochemical markers are needed to separate them into their respective types.

Disclosures: T.L. Garrett: None. **C.L. Wintermute:** None. **M.E. Moran:** None. **S.M. Elbasiouny:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.04/D28

Topic: C.06. Neuromuscular Diseases

Support: SMA Europe Postdoctoral Fellowship

Title: A network-biology approach for the development of combinatorial treatments for the motoneuron-disease spinal muscular atrophy (SMA)

Authors: *N. HENSEL[1,2], I. M. TAPKEN[1], F. CIERI[3], K. JUNG[4], E. DI SCHIAVI[3], P. CLAUS[1,2];
[1]Neuroanatomy and Cell Biol., Hannover Med. Sch., Hannover, Germany; [2]Ctr. for Systems Neurosciences (ZSN), Hannover, Germany; [3]Inst. of Biosci. and BioResources, IBBR, CNR,

Naples, Italy; [4]Inst. for Animal Breeding and Genet., Univ. of Vet. Med. Hannover, Hannover, Germany

Abstract: Spinal Muscular Atrophy is a motoneuron disease which is caused by loss-of-function mutations or deletions of the Survival of Motoneuron gene 1 (*Smn1*). However, all human individuals possess a very similar gene coding for the same protein, the *Smn2* gene. The critical difference between both genes is a C to T transition within an exonic splice enhancer region. The *Smn2* pre-mRNA is insufficiently spliced producing only low amounts of functional full-length SMN-protein compared to the *Smn1* gene. Strategies to enhance the protein production from the *Smn2*-gene have been followed for a long time in experimental SMA-models. In 2017, those efforts led to the approval of the first SMA-specific treatment -Nusinersen or Spinraza© a splice-correcting Antisense Oligonukleotide (ASO), a milestone for the SMA-community. Although resulting in dramatic benefits with regard to survival and motor functions, there is a substantial number of non-responders. Since SMN has already been enhanced in Spinraza©-treated patients other complementary approaches are needed. Those are termed SMN-independent approaches. Previously, we and others reported a number of changed signaling pathways with differing potentials as SMN-independent treatments targets. However, signaling pathways act in a network and this network character has been neglected in the SMA-field so far. Thus, we present novel data on a systems-biology approach towards altered signaling in SMA. We used pre-symptomatic and onset Taiwanese SMA-mice to generate a network of changed pathways allowing an informed decision for highly connected targets. Among those, we identified B-Raf, which was pre-symptomatically down-regulated in SMA-mice. This down-regulation was located to lower motoneurons in the spinal cord. Importantly, we could rescue a *C.elegans* SMA-model with a neuronal over-expression of a B-Raf orthologue. B-Raf is a neuronal Raf family kinase which propagates neurotrophic factor signaling in motoneurons. Thus, our findings may point towards a novel mechanism of motoneuron-degeneration which could be used as an SMN-independent AAV-based approach for future combinatorial treatment regimens.

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Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.05/D29

Topic: C.06. Neuromuscular Diseases

Support: NIH-NINDS, grant #: NS091836

Title: CyPPA effects on SK channels in $SOD1^{G93A}$ mouse model

Authors: *M. M. MURPHY[1], T. L. GARRETT[1], S. M. ELBASIOUNY[1,2];
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Abstract: Amyotrophic Lateral Sclerosis, also known as Lou Gehrig's disease, is a fatal, progressive, neurodegenerative disease with no cure and limited treatment options of modest effects. One of the many hallmarks of the disease is motoneuron degeneration. Within the cell membrane of motoneurons are small conductance calcium-activated potassium channels (SK channels) that function to mediate medium afterhyperpolarization period. SK channels, specifically SK2 and SK3 isoforms, have been shown through immunohistochemistry in our lab to be affected in the $SOD1^{G93A}$ mouse model when compared to wild-type littermates. This study investigated whether neonatal administration of CyPPA had effects on SK channels in the $SOD1^{G93A}$ mouse model at early and late pathological time points. Immunohistochemistry was performed at two time points, postnatal day 21 (P21) and postnatal day 90 (P90). It was found that both isoforms of SK channels increased in expression in wild-type and $SOD1^{G93A}$ at both time points, P21 and P90. CyPPA did target SK channels in the diseased animal and had lasting effects on SK channels by increasing their expression long after administration was ceased. This therefore is suggestive that CyPPA administration could have a beneficial effect on the function of diseased motoneurons.

Disclosures: M.M. Murphy: None. **T.L. Garrett:** None. **S.M. Elbasiouny:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.06/D30

Topic: C.01. Brain Wellness and Aging

Support: CONACYT Grant CB-254728

Title: Prolactin protection against oxidative and hypoxic stress in hippocampal neurons

Authors: F. MACÍAS[1], M. ULLOA[1], R. M. AROÑA[1], C. CLAPP[1], G. MARTÍNEZ DE LA ESCALERA[1], ***E. ARNOLD**[2];
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Abstract: Oxidative stress (OS) is an imbalance in favor of pro-oxidants, like reactive oxygen species (ROS), against antioxidants. This process is commonly associated with a disruption in the redox machinery of the cell and with macromolecular damage. OS is related with the

occurrence and progression of neurodegenerative diseases such as Alzheimer´s disease, Parkinson´s disease, Huntington´s disease and hypoxic-ischemic injury. Thus, efforts have been done to find antioxidant-protective factors that could diminish the cytotoxic effect of ROS. Prolactin (PRL) is a pituitary hormone, mainly known for its actions in lactation and reproduction. In the brain PRL acts as a neuroprotective factor. Some studies have reported that PRL protects hippocampal cells against excitotoxicity *in vitro* and *in vivo.* Furthermore, PRL protects retinal pigment epithelium cells against oxidative damage induced by hydrogen peroxide (H_2O_2). Therefore, in this work we investigated whether PRL protects hippocampal neurons under OS conditions induced by H_2O_2 and hypoxic stress induced by cobalt chloride ($CoCl_2$). Primary cultures of hippocampal neurons were isolated from the brain of E16 mice and incubated for during 12 days *in vitro* (DIV). The cultures were characterized by immunocytochemistry for the expression of the specific neuronal marker beta-III-tubulin. Purity and maturity of neuronal cultures was assessed by the expression of GFAP (astrocyte marker); sinaptophysin, vGlut1 and PSD95 (synapsis markers); and PRL receptor at DIV 4, 10 and 12 by qRT-PCR. The highest expression levels of the synaptic markers as well as the expression of the PRL receptor were observed at DIV10. At this time the cultures were incubated with increasing concentrations of H_2O_2 (25 to 225 M) or $CoCl_2$ (100 to 600 M), to determine LD_{50}. Some cultures were incubated with increasing concentrations of PRL (1 nM to 100 nM) at DIV9, before the challenge with H_2O_2 or $CoCl_2$ (LD_{50}). The treatment with 50 nM and 100 nM of PRL prevents the reduction of the cell viability induced by H_2O_2 but not by $CoCl_2$, as determined by the MTT assay. These results suggest that PRL is a potential neuroprotective factor against oxidative stress.

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Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.07/D31

Topic: C.06. Neuromuscular Diseases

Support: The Giving Back Fund

Title: Gene-replacement strategy for IRF2BPL, an intronless gene intolerant to variations

Authors: *S. SINHA RAY, S. LIKHITE, C. DENNYS-RIVERS, R. RODRIGO, X. ZHANG, M. SCHWARTZ, N. WEIN, K. C. MEYER;
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Abstract: The Interferon Regulatory Factor 2 Binding Protein Like (IRF2BPL) is an intronless gene that encodes a member of the IRF2BP family of transcriptional regulators and is ubiquitously expressed. The protein consists of two highly conserved domains, a coiled-coil DNA binding zinc finger domain at the amino terminus and a C3HC4-type RING finger domain at the carboxy-terminus. The function of the IRF2BPL protein is currently unknown. The gene was initially proposed to play a role in the initiation of puberty in female rodents and non-human primates. More recently, mutations in this gene were associated with neurodegenerative and neurological phenotypes in children, indicating that the gene might play an important role in both development and neuronal maintenance. Specifically, nonsense variants of the IRF2BPL gene lead to severe neurodevelopmental regression. We have established patient fibroblast cell lines having nonsense variants in the IRF2BPL gene that result in the truncation of its RING finger domain. To study the phenotype in cells from the nervous system, we are using an established direct reprogramming method to generate induced neuronal progenitor cells (iNPCs) that can then be further differentiated into astrocytes, neurons and oligodendrocytes. In addition to the cell lines, we established multiple AAV based gene therapy tools to modulate the expression of the protein. Using this *in vitro* system and the AAV vectors, we are able to study the disease mechanism and test potential therapeutic strategies.

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Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.08/D32

Topic: C.01. Brain Wellness and Aging

Support: 5R01MH018501-48

Title: Inositol polyphosphate multikinase is a regulator of transsulfuration pathway

Authors: *R. TYAGI[1], S. H. SNYDER[1], B. D. PAUL[2];
[1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Inositol polyphosphate multikinase (IPMK) is one of the members of inositol phosphate kinase family that generates inositol polyphosphates. IPMK possesses inositol phosphate kinase (IP3-kinase) as well as phosphatidylinositol kinase (PI3-kinase) activities. IPMK is a pleiotropic protein and non-catalytically regulates mammalian target of rapamycin complex 1 (mTORC1), serum response factor, p300, and tumor suppressor protein p53. We report that IPMK regulates expression of cystathionine γ- lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST), enzymes involved in Hydrogen sulfide production. Protein levels of

CSE and MST are increased in IPMK null fibroblasts as compared to wild type fibroblast cells. Regulation of CSE and MST is independent of catalytic activity of IPMK. IPMK regulates CSE expression at the transcriptional level. Since cystathionine β-synthase (CBS) is expressed at relatively lower levels as compare to CSE in fibroblasts, CSE is the major source of cysteine generation from cystathionine in mouse fibroblasts. Lysates prepared from IPMK null fibroblasts produced more cysteine as compared to wild type cells. Hydrogen sulfide levels are also increased in IPMK null fibroblasts. Expression of CSE is inducible upon condition such as lipopolysaccharides (LPS), and inflammation mediated by tumor necrosis factor α (TNF-α). We propose that IPMK acts as a repressor of CSE expression.

Disclosures: R. Tyagi: None. **S.H. Snyder:** None. **B.D. Paul:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.09/D33

Topic: C.06. Neuromuscular Diseases

Support: European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778003

Title: Human 3D cell culture systems to model and elucidate spinal muscular atrophy pathology and treatment

Authors: *S. CORTI[1], **I. FARAVELLI**[1], P. RINCHETTI[1], S. MANCINELLI[2], L. MAPELLI[3], G. FOROTTI[4], M. RIZZUTI[4], C. CORDIGLIERI[5], L. CALANDRIELLO[4], S. TAMANINI[1], N. BRESOLIN[1], G. P. COMI[1], S. LODATO[2], M. NIZZARDO[1];
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Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disorder that represents the leading cause of death due to genetic disease during childhood. SMA results from mutations in the Survival Motor Neuron (SMN) gene coding for SMN, a ubiquitously expressed protein that plays a fundamental role in RNA processing. The optimization of available therapies and the development of complementary therapeutic approaches to SMA requires a deeper understanding of SMN pathophysiology in reliable models. We herein present a new model of SMA pathology in 3D human central nervous system (CNS) organoids. CNS organoids can be obtained from induced pluripotent stem cell (iPSCs) differentiated with a free-floating 3D culture method. Brain and spinal cord-like organoids closely recapitulate the endogenous developmental program, presenting well-defined and interdependent neuronal regions that reproduce the

integrated connection of the CNS. We generated iPSCs from fibroblasts of SMA patients and healthy controls and developed CNS organoids, which gave rise to an early cerebral cortex-like formation containing progenitor cells and more mature neural subtypes. We performed immunohistochemical analyses and single-cell RNAseq to confirm the organoids differentiation state. Electrophysiological studies were also undertaken to study function and activity. Using a modified protocol to promote neural caudalization and ventralization, we also derived spinal cord-like organoids, which offer an unprecedented powerful tool to elucidate early MN pathology. Spinal cord-like organoids were also transplanted into a murine model to ensure proper maturation and to allow studies on a broader time-frame. Preliminary results demonstrated that SMA organoids exhibited a significant alteration in their electrophysiological activity compared to those derived from healthy controls. SMA organoids transplanted into the mouse model integrated with the host spinal cord and remained vital in the medium-long term. Treatment of SMA organoids with a second-generation optimized anti-sense oligonucleotide significantly increased SMN levels. Our data support the use of CNS organoids as an innovative *in vitro* platform for studying neurological disease pathogenic mechanisms and to test potential therapeutic strategies.

Disclosures: **S. Corti:** None. **I. Faravelli:** None. **P. Rinchetti:** None. **S. Mancinelli:** None. **L. Mapelli:** None. **G. Forotti:** None. **M. Rizzuti:** None. **C. Cordiglieri:** None. **L. Calandriello:** None. **S. Tamanini:** None. **N. Bresolin:** None. **G.P. Comi:** None. **S. Lodato:** None. **M. Nizzardo:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.10/D34

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant MH18501

Title: Bilirubin, a component of a metabolic traffic signal, links heme metabolism to neuronal stress signaling

Authors: ***B. D. PAUL**[1], C. VASAVDA[1], R. KOTHARI[1], R. TOKHUNTS[2], T. W. SEDLAK[3], S. H. SNYDER[1];
[1]Johns Hopkins Univ. Sch. of Med., Batimore, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Bilirubin is one of the most commonly measured peripheral metabolites, yet its precise role in the brain is not well understood. Bilirubin is synthesized by Biliverdin reductase (BVR) from biliverdin, which in turn is derived from heme. BVR exists as two isoforms, BVR-A

and BVR-B, of which BVR-A is enriched in the brain. In addition to its role in bilirubin generation, BVR-A is a kinase and transcription factor, and participates in several signaling pathways. Besides, these activities, the BVR pathway plays significant roles in maintenance of redox balance in cells. Cell culture studies have shown that depletion of *BlvrA*, the gene encoding BVRA causes elevated oxidative stress and associated cellular damage, however, the effects of *BlvrA* depletion in the brain has not been well studied. Using *BlvrA* knockout mice, we show that BVRA is the major enzyme responsible for bilirubin production. The gall bladders of these mice are green due to accumulation of biliverdin and inability to produce bilirubin. Depletion of bilirubin is associated with elevated superoxide production in the brain and altered cytoprotective response to stress stimuli. These mice are defective in several antioxidant defense pathways. *BlvrA* mice also display a variety of behavioral abnormalities including cognitive deficits. We further show that bilirubin is a potent and direct superoxide scavenger both *in vitro* and *in vivo*, which accounts for its role in maintenance of redox balance. Treatment of mice with NMDA, an excitotoxin, results in increased cell death and larger lesions in *BlvrA* knockout mice. NMDA receptor activation is known to elevate superoxide production via the NADPH oxidases, enzymes which are known to be inhibited by the BVR-bilirubin pathway. Taken together, our findings uncover important roles for BVR in neuroprotective processes. Modulating the BVR/bilirubin pathway may offer therapeutic benefits in neurodegenerative diseases involving redox imbalance.

Disclosures: B.D. Paul: None. **C. Vasavda:** None. **R. Kothari:** None. **R. Tokhunts:** None. **T.W. Sedlak:** None. **S.H. Snyder:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.11/D35

Topic: C.01. Brain Wellness and Aging

Support: JSPS Grant-in-Aid for JSPS Research Fellow (18J21936)

Title: Enhancement of glucose uptake to maintain ATP levels in the brain neurons and calorie restriction synergistically antagonize the aging in *drosophila*

Authors: *M. OKA[1], E. SUZUKI[2,3], K. M. IIJIMA[4,5], K. ANDO[1];
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Abstract: Aging impairs cognitive functions and increases the risks of neurodegenerative diseases. Accumulating evidence suggests that disruption of energy metabolism underlies these

age-associated changes. The human brain is estimated to consume over 50% of total glucose in the body, and glucose metabolism is thought to play a critical role in the brain aging. However, how energy metabolism is changed in brain neurons during aging, and how such changes contribute to the age-related decline in neuronal function, are not fully understood. Here we report that changes in glucose metabolism and ATP levels in the brain neurons underlie organismal aging in Drosophila. Glucose levels reduce in heads but not in bodies during aging. qRT-PCR revealed that expression levels of glucose transporter 1 reduce during aging in fly brains. By using transgenic flies expressing genetically encoded fluorescent ATP biosensor, we found that ATP levels reduced in the soma of neurons in the mushroom body during aging. Enhancement of glucose uptake by neuronal overexpression of a glucose transporter was sufficient to suppress the age-dependent declines in ATP levels and locomotor functions. Interestingly, CR treatment also suppressed the age-dependent reduction in ATP levels in brain neurons, suggesting that maintaining ATP levels in aged brains may be one of the common pathways to counteract organismal aging. Finally, we found that increased glucose uptake in brain neurons and CR synergistically extended the life span. Our results suggest that a reduction in neuronal ATP levels underlies the aging processes, which can be antagonized via CR or enhanced uptake of glucose to neurons. Our results also suggest that local management of glucose supply in brain neurons and control of circulating glucose levels may synergistically enhance functional integrity against aging.

Disclosures: **M. Oka:** None. **E. Suzuki:** None. **K.M. Iijima:** None. **K. Ando:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.12/D36

Topic: C.01. Brain Wellness and Aging

Support: W.M. Keck Foundation

Title: Resting state brain activity optimizes a trade-off between coordinated firing activity, task adaptability, and metabolic cost

Authors: *C. WEISTUCH, A. AMGALAN, S. F. SULTAN, K. DILL, L. MUJICA-PARODI; Stony Brook Univ., Stony Brook, NY

Abstract: What is the relationship between cognitive function, neural firing patterns, and physical constraints such as energy? We argue that healthy brains endure large energetic costs in order to optimize a balance between coordinated firing activity and task adaptability; loss of this balance leads to neurocognitive impairment. Using ultra-high field (7T) fMRI in conjunction with the Ising model from statistical physics, we show that this balance is disrupted when brain

metabolism is impaired through age (n=636). Additionally, we show that this signature of age-related cognitive decline is partially reversed by the ketogenic diet (n=12), further establishing the role that energy and insulin-resistance have on cognitive impairment. Taken together, our results provide evidence for a novel organizing principle of the brain, as well as, demystify the role energy plays in organizing functional activity.

Disclosures: C. Weistuch: None. **A. Amgalan:** None. **S.F. Sultan:** None. **K. Dill:** None. **L. Mujica-Parodi:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.13/D37

Topic: C.06. Neuromuscular Diseases

Support: grant from Huntington's Disease Foundation

Title: Crosstalk between DNA repair pathways in Huntington's disease

Authors: *A. PLUCIENNIK, H. ABDULLAH;
Biochem. and Mol. Biol., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Huntington’s disease (HD) is a neurodegenerative disorder caused by an expansion of a CAG repeat tract within the huntingtin (*HTT*) gene, leading to neuronal death primarily in the striatum and the cortex. The CAG repeat is highly unstable, both intergenerationally and in somatic cells. HD patients display a high degree of age-dependent somatic expansion of the CAG repeat in the striatum, although such expansions also occur in other tissues. Recent genome-wide association studies of HD patients have revealed the existence of genetic modifiers of the age of onset of the disease; these include several genes involved in DNA repair, and in particular DNA mismatch repair (*MSH3, MLH1, PMS2, PMS1*). In addition, studies in mouse models of HD have revealed that genetic knockout of the DNA mismatch repair genes, *Msh2, Msh3, Mlh1, or Mlh3* reduces somatic instability of CAG repeats in the striatum. These data suggest that somatic expansion of CAG repeats is likely linked to striatal neuronal loss, and onset of disease symptoms in HD patients. Nevertheless, the role of mismatch repair proteins and other accessory factors in mediating CAG repeat expansion remains poorly understood. As a first step towards understanding these processes, we have initiated interactomic studies aimed at characterizing the protein assemblies that recognize and process CAG extrusions (structures formed by DNA strand slippage within CAG repeat tracts). Our proof of concept pull-down assays from HeLa nuclear extracts recovered not only known proteins that might be expected to associate with CAG extrusions like MutSbeta (MSH2/MSH3 heterodimer) and PCNA but also additional novel proteins. We have also developed a proximity biotinylation (TurboID)-based

assay in human HEK293 cells to evaluate transient and stable protein assemblies on DNA containing CAG extrusions, with a view to eventually applying this approach to cells of neuronal origin. We anticipate that these complementary interactome approaches will shed light on the pathways involved in processing CAG extrusions, and enable a dissection of the functions of individual proteins in driving CAG repeat expansion.

Disclosures: A. Pluciennik: None. **H. Abdullah:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.14/D38

Topic: C.01. Brain Wellness and Aging

Support: NIA R01 AG033036
NIA R01 AG055449
NIH P50 AG05144
NIH T32 AG00242

Title: Iron accumulation is selectively associated with brain volume and vascular white matter health

Authors: *C. E. BAUER, V. ZACHARIOU, E. SEAGO, B. T. GOLD;
Neurosci., Univ. of Kentucky, Lexington, KY

Abstract: Non-heme intracellular iron is critical to normal cellular function, however it is also a powerful oxidizer that creates reactive oxygen species. Aging is often associated with iron accumulation in subcortical brain structures, which disrupts cellular functions through oxidative stress and can lead to inflammation, demyelination, and ultimately neurodegeneration and atrophy. Limited evidence also suggests that iron accumulation may be associated with cerebrovascular disease, possibly through iron-mediated weakened vasculature. In this study, we investigated the possible association between iron deposition in basal ganglia, brain atrophy, and cerebrovascular disease using magnetic resonance imaging (MRI). Forty-one healthy older adults (ages 67-85) were recruited through the UK Sanders-Brown Center on Aging. We measured iron accumulation in the caudate and putamen using MRI-derived quantitative susceptibility mapping (QSM). T1-weighted images were segmented using FreeSurfer; intracranial volume-corrected grey and white matter and ventricular volume were calculated. Cerebrovascular disease was quantified as white matter hyperintensities (WMHs), or high-intensity signal seen in white matter on T2-weighted images, and were defined as voxels 3.5 standard deviations above mean white matter signal intensity value. Linear regression models with QSM values as the predictors and volumetric measures and WMHs as the dependent variables were conducted in SPSS controlling

for age. Grey matter volume declined as both caudate (p=0.029) and putamen (p=0.054) QSM values increased. Furthermore, these QSM values were positively associated with deep WMH volume (p=0.021; p=0.04). Further investigation based on a lobar analysis revealed that basal ganglia QSM values elevated with only the occipital deep WMHs (p=0.005; p=0.015), but not deep WMHs in any other lobe. Separation of participants into those with a history of hypertension and those without indicated that iron levels in hypertensive individuals were elevated in both caudate (p=0.035) and putamen (p=0.016) regions. Our results support the link between basal ganglia iron deposition, whole brain grey matter atrophy, decreased vascular brain health, and hypertension. Excessive iron accumulation may be involved in early disease processes before the appearance of clinical symptoms.

Disclosures: C.E. Bauer: None. **V. Zachariou:** None. **E. Seago:** None. **B.T. Gold:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.15/D39

Topic: C.01. Brain Wellness and Aging

Support: NIH K08 NS057824
BBH/NARSAD

Title: The glutathione cycle shapes synaptic glutamate transmission - Translational opportunities

Authors: *T. W. SEDLAK[1], B. D. PAUL[2], M. KOGA[3], A. SAWA[4], S. H. SNYDER[5];
[1]Johns Hopkins, Baltimore, MD; [2]The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Dept. of Psychiatry, Natl. Def. Med. Col., Tokorozawa, Japan; [4]Dept. of Psychiatry, Johns Hopkins Univ., Baltimore, MD; [5]Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Glutamate is the most abundant excitatory neurotransmitter, present at the bulk of cortical synapses, and participating in many physiologic and pathologic processes ranging from learning and memory to stroke. The tripeptide, glutathione, is one third glutamate and present at up to low millimolar intracellular concentrations in brain, mediating antioxidant defenses and drug detoxification. Because of the substantial amounts of brain glutathione and its rapid turnover under homeostatic control, we hypothesized that glutathione is a relevant reservoir of glutamate, and could influence synaptic excitability (Sedlak et al, PNAS 2019). We find that drugs which inhibit generation of glutamate by the glutathione cycle elicit decreases in cytosolic glutamate and decreased miniature excitatory post synaptic potential (mEPSC) frequency. In contrast, pharmacologically decreasing the biosynthesis of glutathione leads to increases in cytosolic glutamate and enhanced mEPSC frequency. The glutathione cycle can compensate for

decreased excitatory neurotransmission when the glutamate-glutamine shuttle is inhibited. Glutathione may be a physiologic reservoir of glutamate neurotransmitter. We also report that the phytochemical sulforaphane, increases glutathione in rat cortical neurons and in circulating leukocytes of normal human participants.

Disclosures: T.W. Sedlak: None. **B.D. Paul:** None. **M. Koga:** None. **A. Sawa:** None. **S.H. Snyder:** None.

Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.16/D40

Topic: C.06. Neuromuscular Diseases

Title: Single-cell RNA-seq analysis of Batten genes in the retina and AAV targeting approaches

Authors: *M. K. SCHWARTZ[1,2], S. B. LIKHITE[2], A. CAMPBELL[1], I. PALLAZZO[1], R. J. PINEDA[2], P. R. MORALES[3], A. FISCHER[1], K. C. MEYER[2];
[1]The Ohio State Univ., Columbus, OH; [2]The Res. Inst. at Nationwide Children's Hosp., Columbus, OH; [3]The Mannheimer Foundation, Inc., Homestead, FL

Abstract: Batten Diseases are severe neurodegenerative disorders leading to progressive loss of cognitive and motor function. Several groups are currently developing gene therapy strategies to address the unmet need of treatment options for these patients. The vision loss that occurs in most Batten Disease forms is a particular challenge for treatment as many commonly used delivery routes are not efficient in targeting the retina. Moreover, the underlying cause and mechanisms of retinal degeneration in Batten disease are currently unknown. Thus, it is unclear which cell types need to be targeted in the retina for an effective gene therapy and whether the cell types are the same across all Batten Diseases or not. In this study, we evaluated the expression levels of various Batten Disease genes in retinal cell types of mice and non-human primates using single cell RNA sequencing. We found that the patterns of expression are similar between mice and non-human primates, but substantially differ between various Batten Disease genes. In order to achieve targeting of different cell types in the retina, we evaluated several AAV serotypes, promoters and injection routes. To reduce variation due to vector preparation, all vectors were produced simultaneously and by the same source. To date, methods to determine AAV vector concentrations vary greatly between laboratories. It has been shown by multiple groups that quantitative PCR assays might not be accurate enough to distinguish smaller differences in vector concentration. These factors might confound comparison of vector efficiency. Therefore, to ensure an accurate comparison, we used digital droplet PCR detecting a common sequence in all our vectors to determine vector concentration. Our results show that the injection route impacts the targeting of different cell layers in the retina. Also, different

promoters have different expression efficiencies in different cell types within the retina. Moreover, we dosed 4-year old and a 10-year old non-human primate intrathecally with AAV9-GFP and analyzed expression throughout the nervous system as well as targeting of the retina and the specific cell types in the retina. We show with immunofluorescence staining as well as qPCR and ddPCR that intrathecal dosing allows targeting of the retina in these large animals. Moreover, the cell types in the retina show different targeting efficiency with this delivery route. Our findings will further improve future Batten Disease gene therapy treatments and might impact decisions on viral vectors, promoter and delivery routes for other retinal diseases as well.

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Poster

045. Mechanism Underlying Neurodegenerative Disease

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 045.17/D41

Topic: C.06. Neuromuscular Diseases

Title: Induced pluripotent stem cells (iPSCs) and iPSCs-derived motor neurons (MNs) obtained from patients with Brown-Vialetto-Van Laere (BVVL) syndrome reveal alterations of redox status, mitochondrial features and cytoskeletal organization

Authors: *A. NICEFORO[1,2,4], S. PETRINI[3], F. COLASUONNO[1], Z. ABBASZADEH[3], V. D'ORIA[3], E. BERTINI[2], S. MORENO[1], L. QIANG[4], P. W. BAAS[4], C. COMPAGNUCCI[2]; [1]Dept of Sci., Univ. Roma Tre, Rome, Italy; [2]Dept. of Neuroscience, Lab. of Mol. Med. IRCCS, [3]Confocal Microscopy Core Facility, Children's Res. Hosp. Bambino Gesù, Rome, Italy, Rome, Italy; [4]Dept. of Neurobio. and Anat., Drexel Univ., Philadelphia, PA

Abstract: Brown-Vialetto-Van Laere syndrome (BVVL) is a rare, progressive, childhood-onset disorder characterized by progressive ponto-bulbar palsy associated with neurodegeneration of spinal Motor Neurons (MNs)[1,2]. The disease is caused by loss-of-function mutations in two human Riboflavin Transporters: RFT2 and RFT3. Riboflavin (vitamin B2), as a precursor of FMN and FAD, is a crucial micronutrient for normal cellular functions, particularly involving energy metabolism pathways driven by flavoproteins[3].Proteomic studies suggest an interaction between riboflavin transporters and tubulins; we further investigated this aspect by Fluorescence-lifetime imaging microscopy (FLIM) and Förster resonance energy transfer (FRET) analysis in control iPSCs and iPSCs-derived MNs. Our results show that the lifetime of RFT2 transporter decreases in the presence of α-tubulin, indicating a direct interaction between RFT2 and the α-tubulin. Likewise, the lifetime of RFT3 decreases in presence of β and βIII-tubulin.Fibroblasts obtained from BVVL patients were reprogrammed in induced Pluripotent Stem Cells (iPSCs) and after differentiated in MNs, in order to characterize unknown molecular and cellular aspects

of this disease. Patients' iPSCs and iPSCs-derived MNs showed higher levels of superoxide anion and aberrant mitochondrial ultrastructural features *vs.* controls cells, compatible with the concept that riboflavin deficiency affects energy metabolism. Moreover, the treatment with riboflavin and/or other antioxidants (N-acetyl-cysteine, Coenzyme Q, Idebenone, Vitamin C, FMN, Vatiquinone) restored normal mitochondrial morphology and the redox balance in patients' cells. During the differentiation of patients' iPSCs into MNs, deranged organization of cytoskeletal components were observed. Indeed, both immunofluorescence and RT-qPCR analyses showed altered expression and distribution of βIII-tubulin and NFH intermediate filaments and a partial phenotype rescue were observed after riboflavin/antioxidants treatment. Our data support the use of iPSCs to model BVVL syndrome *in vitro* and open the way to future antioxidants-based therapeutic strategies aimed at ameliorating and/or preventing symptoms of BVVL. *1. Sathasivam S, Orphanet J. Rare Dis. 2008, 3:9 2. Van Laere J. Rev Neurol 1966, 115:289-95 3. Barile M et al. J Inherit Metab Dis 2016, 39:545*

Disclosures: A. Niceforo: None. **S. Petrini:** None. **F. Colasuonno:** None. **Z. Abbaszadeh:** None. **V. d'oria:** None. **E. bertini:** None. **S. moreno:** None. **L. Qiang:** None. **P.W. Baas:** None. **C. Compagnucci:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.01/D42

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Breast cancer metastasis to the brain: Biomarkers of drug resistance

Authors: *A. M. FLOREA[1], D. BUSSELBERG[2];
[1]Federal Inst. For Risk Assessment (bfr), Berlin, Germany; [2]Weill Cornell Med. Col. In Qatar, Doha, Qatar

Abstract: Breast cancer (BC) is a main cause of death in woman worldwide. Over 450,000 BC deaths and 1.3 million cases of invasive BC are recorded each year while BC is the second-leading cause of metastatic disease in the central nervous system (CNS). A challenging problem for the clinical oncologists represents the invasive, recurrent, and chemotherapy resistant BC that results in brain metastases such as the triple-negative breast cancer (TNBC).
Drug resistant breast tumor cells are able to turn "on" drug resistance mechanisms that are currently not fully understood. These cells are able to diminish the effects of chemotherapy due to changes in gene expression and cellular functions which play a role in cancer related processes including cancer progression and its response to chemotherapy. Molecular research that aims to find possible weaknesses in TNBC is the most important today. Here we review the current literature that addresses the changes occurring upon TNBC acquired drug resistance upon

chemotherapy. These specific molecular targets might be used in clinics for a more efficient diagnostic and targeted therapy.

Disclosures: A.M. Florea: None. **D. Busselberg:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.02/D43

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Cisplatin's dual-effect of the circadian clock triggers proliferation and apoptosis

Authors: *D. BUSSELBERG, Z. SADIQ;
Weill Cornell Med. Col. In Qatar, Doha, Qatar

Abstract: The circadian clock, which generated the internal daily rhythm, can be disrupted in a variety of ways. Multiple environmental factors result in circadian disruption mostly by altering the release of melatonin. Also, anti-cancer drugs including cisplatin affects the circadian clock. Cisplatin modulates the circadian clock through two mechanisms: 1) the circadian clock control of DNA excision repair and 2) the effect of circadian clock disruption on apoptosis. Cisplatin can activate various classified molecules, including DNA damage-recognition factors, DNA repair factors and transcription factors involved in drug resistance and cisplatin-induced signal transductions. These factors mutually interact and might be altered by DNA damage. Hence, these molecular interactions are closely involved in cell proliferation and damage-induced apoptosis. Cisplatin has a dual-effect on circadian genes: upregulation of CLOCK expression causes an increase in proliferation but upregulation of BMAL1 expression causes an increase in apoptosis. As such, the interference of clock genes by cisplatin can have opposing carcinogenic effects. Melatonin and intracellular Ca^{2+} also have a dual-effect on cell proliferation and apoptosis and can disrupt the circadian clock. Overall, anti-cancer drugs like cisplatin interfere with components of the circadian clock causing either an increase or decrease in apoptosis and cell proliferation. These drugs, in combination with each other or with other types of agents, may have powerful anti-cancer effects, but they may also have unintended pro-cancer effects.

Disclosures: D. Busselberg: None. **Z. Sadiq:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.03/D44

Topic: C.10. Brain Injury and Trauma

Support: FI-DGR-2016 Grant

Title: RNA-seq analysis of DRG sensory neurons and satellite glial cells in a mouse model of cisplatin-induced peripheral neuropathy: An exploratory study

Authors: *A. CALLS, E. UDINA, X. NAVARRO, R. VELASCO, J. BRUNA;
Inst. of Neurosciences, Univ. Autònoma de Barcelona, Bellaterra, Spain

Abstract: Cisplatin is a very effective cytostatic drug for the treatment of cancer. However, oncologic patients treated with cisplatin often develop a cisplatin-induced peripheral neuropathy (CIPN), which results in a reduction of drug dosage or even cessation of chemotherapy treatment. Therefore, it is necessary to understand the neurotoxic mechanisms of cisplatin and identify molecular pathways for neuroprotective interventions. On the other hand, the potential role of satellite glial cells (SGC) in the occurrence of CIPN has never been explored. In this study, we aimed to determine the exact molecular pathways involved in the development of CIPN. To reach this proposal, we performed a single-cell RNA-sequencing (scRNAseq) of dorsal root ganglia (DRG) cell populations (including neurons and SGC) from control and CIPN-developing mice. We have developed a new mice model of CIPN. Cisplatin is intraperitoneally injected to Balb/C female mice once a week at a dose of 7mg/kg until reaching a total cumulative dose of 42mg/kg. Nerve conduction studies show a progressive reduction in sensory nerve amplitudes in both digital and caudal nerves along cisplatin treatment. On the other hand, cisplatin-treated mice develop mechanical allodynia by means of a progressive decrease in their withdrawal threshold to mechanical stimuli. At the end of the treatment, animals receiving cisplatin have similar amount of myelinated nerve fibers in the sciatic nerve and no differences in the density of intraepidermal nerve fibers compared to control animals (n=10/group). In contrast, DRG neurons of cisplatin-treated mice present large and flat mitochondria, prominent dilatations in their endoplasmic reticulum system, and autophagosome-like vesicles. For the scRNAseq, all DRG of control and CIPN-developing mice (n=5/group) were dissected two weeks after the last cisplatin administration. We first isolated individual DRG cells by single cell-sorting. Then, cell type clusterization was performed by considering the relative expression of well-known cellular markers of neurons (*Tubb3, Rbfox3, Eno2, Gap43*), endothelial cells (*Flt1, Pecam1, Cd34*), macrophages (*Cd45, Cd68, Adgre1*), fibroblasts (*100a4, Col1a2*)and SGC (*Glul, Slc1a3, Cdh19*). A total of 71 neurons and 99 SGC were used for the analysis. Both neurons and SGC of CIPN-mice have an up-regulation of p21 expression when comparing with

controls. SGC exposed to cisplatin also up-regulates H3.3B histone. Western blot of whole DRG lysates shows an up-regulation of p53 protein in cisplatin-treated mice. All these proteins are described to be up-regulated after DNA damage, thus giving strength to the CIPN-animal model we have developed.

Disclosures: A. Calls: None. **E. Udina:** None. **X. Navarro:** None. **R. Velasco:** None. **J. Bruna:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.04/D45

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIDA Intramural Research Program

Title: Depletion of endoplasmic reticulum calcium by neuronal activation triggers redistribution of resident ER proteins

Authors: *B. K. HARVEY, L. FORTUNO, K. A. TRYCHTA, C. T. RICHIE, A. M. DOSSAT; NIDA - NIH, Baltimore, MD

Abstract: Elevated levels of synaptic glutamate can produce neuronal excitotoxicity, which occurs in conditions such as epilepsy and stroke. The role of calcium ($^{Ca2+}$) at the plasma membrane and within the cytoplasm towards the development of excitotoxicity is well established. However, less is known regarding the contribution of endoplasmic reticulum (ER)-sourced $^{Ca2+}$ on this pathophysiological process. Our laboratory recently discovered that ER resident proteins which contain an ER retention sequence (ERS) at their C-terminus are secreted in response to depletion of ER calcium as part of a process termed “exodosis.” Here, we use a luciferase reporter with an ERS tail to monitor exodosis caused by glutamate receptor activation in rat primary cortical neurons. Glutamate caused a time and dose-dependent release the luciferase reporter. Antagonism of ER $^{Ca2+}$ channels blunted the effects of GluR agonists, supporting that neuronal activation promotes $^{Ca2+}$ efflux and exodosis from this organelle. Overexpression of ERS receptors (i.e. KDEL receptors) attenuated the effects of GLuR agonists to promote ERS protein release, indicating the effects of these GluR agonists to induce exodosis can be blunted by KDEL receptors. Collectively, our data indicate that glutamatergic activation can disrupt ER calcium homeostasis and promote exodosis phenomenon may provide insight into novel druggable targets for disorders associated with excitoxicity.

Disclosures: B.K. Harvey: None. **L. Fortuno:** None. **K.A. Trychta:** None. **C.T. Richie:** None. **A.M. Dossat:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.05/D46

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: MRP Grant MRP-092-17X
HMRF Grant 14151281

Title: Electrical stimulation boosts differentiation and maturation of neural stem cell by improving microenvironment on carbon nanotube scaffold

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Abstract: Electrical stimulation (ES) has been applied in the cell culture system to enhance neural stem cells (NSCs) proliferation, migration, and neuronal differentiation. The underlying mechanisms, however, remain thoroughly understood. In this study, we presumed that ES might provide an effective cue for NSCs that combined with a carbon-nanotube topographical cues to initiate biological activations of NSCs. The microenvironment in the neurogenesis of NSCs following ES was a matter of interested research in the current study. Methods:NSCs was isolated from brain of embryonic day 14.5(E14.5) C57 mice. The following experiments can be divided into three groups: PDL, carbon nanotube scaffold (CNTs) and CNTs-ES. After seeding two days, we applied ES to NSCs and the parameters are frequency at 20 Hz, current at 1mA for 2h. Immunfluorescence assay, RT-PCR and Western bolt were used to test the neural markers, such as DCX, Tuj1, MAP2 and NeuN. The neurite growth of differentiated NSCs was constructed by using the neurolucida software. Whole-cell patch clamp recording was performed using an EPC-9 amplifier. Calcium imaging was measured with the indicator Fura-2 AM in living cell system. RNA-seq was used to detect the change of transcriptional level during the development of NSCs to neurons, cytokines protein arrays and hierarchical clustering analysis will be done to check the change of microenvironment.Results:ES could promote elongation of neurite growth from NSCs compared with the CNTs condition. ES also increased the expression of neural markers during the differentiation and maturation of NSCs, including DCX, Tuj1, MAP2 and NeuN on day 7 compared with no-ES group. ES could enhance the expression of PSD95 and BDNF on day 14, which meant the differentiated neurons of CNTs-ES group could be more mature and better functionality. Then, Electrophysiological results showed that the frequency and peak amplitude of action potential in the CNT-ES group were higher than no-ES CNTs group. ES was performed during live cell monitoring, and intracellular calcium

concentration was rapidly increased and quickly decreased after ES was withdrawn. RNA-seq results and cytokines protein arrays suggested that multiple pathways associated with NSCs growth, differentiation and maturation, especially about the calcium channels. Conclusion: ES could exert a positive effect on the growth, differentiation and maturation of the whole process of NSCs. This article reveals a new mechanisms that ES combined with CNTs regulates the cytokines and trophic factors, thus improved the microenvironment for NSCs differentiation and maturation.

Disclosures: S. Zhongqing: None. **R. Zhu:** None. **B. Niu:** None. **L. He:** None. **K. Chiu:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.06/E1

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: FPU15/00801
SAF-2016-77703

Title: Epigenetic changes after unpredictable mild chronic stress in female SAMR1 and SAMP8: Effects on behavior

Authors: *D. PUIGORIOL-ILLAMOLA, C. GRIÑÁN-FERRÉ, M. PALLÀS;
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Abstract: Cognitive and behavioral disturbances are growing public healthcare issue for the modern society. It has been shown that the environment is crucial in the development of several diseases, as well as compromising healthy aging. Accumulated evidence suggests that stress is an important risk factor in cognitive decline. Besides, life rhythm has changed becoming increasingly stressful. Therefore it is important to study the effects of stressful lifestyle on cognition and its relationship with aging to unveil what challenges we might have to cope with as a society in a not so far future. Likewise, epigenetic alterations are present in brain disorders and are affected by environmental stress. However, little is known about the interaction of stress, learning-memory and epigenetics. Thus understanding the epigenetic modifications that stressful environment triggers in cognition is essential to develop novel therapeutics for age-related cognitive decline. This study aims to determine the effects of a stressful lifestyle in an animal model with accelerated senescence (SAMP8) and compare it with its control strain (SAMR1). Female SAMR1 and SAMP8 mice (n=48) 5 month-old were divided into four groups: SR1-Ct, SR1-UCMS, SP8-Ct and SP8-UCMS. Unpredictable mild chronic stress (UCMS) groups received for one month an UCMS treatment, which consisted of daily applying different stressors

such as food/water deprivation, overnight illumination, sawdust removal, among others. To evaluate the behavior and cognitive performance, several tests as novel object recognition test (NORT) and open field test (OFT) were conducted, followed by molecular analysis of neurodegenerative and epigenetic markers in the hippocampus using Western blot, ELISA, RT-PCR and miRNA assay.Changes in behavioral tests were found leading to reduced recognition and spatial memory in SAMP8, which worsened when UCMS treatment in both strains, as well as a loss of recklessness. Consistent with these results, we found an increased in inflammatory and oxidative stress (OS) markers. Additionally, changes on epigenetic machinery and their epigenetic marks such as DNA methylation (5-mC) and hydroxymethylation (5-hmC) were found. Interestingly, several miRNAs were modified, which were related to neurodegenerative processes.In conclusion, a stressful lifestyle leads to age-related cognitive decline. Besides, UCMS is a feasible intervention to understand the influence of stress on epigenetic mechanisms underlying cognition, opening new avenues for treating cognitive impairment.

Disclosures: D. Puigoriol-Illamola: None. **C. Griñán-Ferré:** None. **M. Pallàs:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.07/E2

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: VIEP BUAP Haciendo ciencia en la BUAP Otoño 2018

Title: Ethanol as a vehicle for the administration of resveratrol prevent oxidative stress in hippocampus of Wistar rats

Authors: *I. CESAR ARTEAGA[1], D. JUÁREZ SERRANO[2], E. BRAMBILA COLOMBRES[3], A. R. NAVARRO CRUZ[4], H. A. RUBIO ZAPATA[5], O. VERA LOPEZ[4], P. AGUILAR-ALONSO[6];
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Abstract: Oxidative stress is the imbalance between endogenous antioxidants and oxidant molecules, these have an unpaired electron in their last valence coat, the most important are the oxygen and nitrogen derivatives. Resveratrol is a natural polyphenol that have antioxidant characteristics reducing oxidative stress, however, the bioavailability of resveratrol is low due to rapid excretion and extensive metabolism, it is known that polyphenols improve their

bioavailability in ethanol presence. Ethanol is a substance widely used in many cultures in different ways including as a vehicle in the pharmaceutical industry. The aim of this work is determine the adequate concentration of ethanol as a vehicle for resveratrol by administration oral. Male wistar rats, 3 moonths old, were divided into the following categories: Control (without treatment), four groups administered with vehicle (2.5, 5, 7.5 y 10% ethanol), and other 4 groups administered with resveratrol 10 mg / kg / day in addition to different concentrations of ethanol. All groups were daily administered orally via for 2 months and all groups have n=3 animals. Posteriorly, the hippocampus was obtained for quantification of nitrite production, MDA + 4-hydroxinonenal, malondialdehyde (MDA), (4-HDA) and enzymatic activity of Catalase and Superoxide Dismutase. The levels of nitric oxide and lipoperoxidation products show a significant decrease at 7.5% of ethanol that when it is administered 10%. So it is concluded that the adequate vehicle concentration to administrate 10 mg resveratrol is 7.5% of ethanol. Resveratrol is a molecule with antioxidant function, when it is administrated with vehicle, it is improves its activity. Despite analyzes carried out, ethanol as a vehicle has adverse effects at a concentration of 7.5%, however, it continue being adecuate for the antioxidant effect obtained from resveratrol.

Disclosures: I. Cesar Arteaga: None. **D. Juárez Serrano:** None. **E. Brambila Colombres:** None. **A.R. Navarro Cruz:** None. **H.A. Rubio Zapata:** None. **O. Vera Lopez:** None. **P. Aguilar-Alonso:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.08/E3

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant K01AG054734
NIH Grant NS064967

Title: Excitotoxic neuronal death inducing megachannel resides in monomeric F_1F_OATP synthase

Authors: *N. MNATSAKANYAN[1], H.-A. PARK[2], J. WU[1], M. LLAGUNO[1], B. MURTISHI[1], P. MIRANDA[1], F. SIGWORTH[1], E. A. JONAS[1];
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Abstract: Mitochondria play a key role in neuronal survival by regulating both energy metabolism and cell death pathways. Activation of mitochondrial permeability transition (mPT) is an important excitotoxic neuronal death pathway used during Alzheimer's disease and other neurodegenerative disorders. The opening of the mitochondrial permeability transition pore

(mPTP) leads to mitochondrial inner membrane permeabilization and dissipation of the mitochondrial membrane potential, followed rapidly by cell death. Despite its vital importance, the exact structural components and mechanisms inducing mPTP opening are poorly understood at the molecular level. Mitochondrial ATP synthase has been shown by us and others to house the mPTP but the oligomeric state of ATP synthase required for channel formation is still being debated. For this study we have purified and reconstituted monomeric ATP synthase from porcine heart mitochondria into small unilamellar lipid vesicles (SUVs) and analyzed its oligomeric state by single-particle electron cryomicroscopy. Here, we present the cryo-EM structure of functionally active monomeric ATP synthase in SUVs at ~16 Å resolution. We also show that this preparation of SUV-reconstituted ATP synthase monomers, when fused into giant unilamellar vesicles (GUVs), forms voltage-gated and Ca^{2+}-activated channels with the key features of mPTP. Based on our findings we conclude that the ATP synthase monomer is sufficient, and dimer formation is not required, for the megachannel activity of ATP synthase. Future in-depth structural analysis of ATP synthase will reveal its "open channel" conformation and may lead to structure-based drug design of specific therapeutic compounds for the treatment of degenerative disorders.

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Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.09/E4

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: MHRD fellowship
University Grants Commission; Grant Number: 6-10/2017(IC)

Title: Exploring the role of glycogen and the synthetic machinery in neuronal stress response

Authors: *A. ONKAR, S. GANESH;
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Abstract: Healthy neurons, though endowed with a functional glycogen synthetic machinery, do not synthesize or store glycogen. However, degenerating neurons evident in several neurodegenerative disorders like Parkinson's, Alzheimer's are known to accumulate glycogen in the brain, either in its normal form or an abnormal form. Earlier reports demonstrated that forced expression of glycogen synthase (GS), the key enzyme involved in glycogen synthesis, results in reduced lifespan in fly and mice, and, conversely, knockdown of GS lead to an increase in the cognitive function and lifespan. Thus, the significance of GS in neurons is not very well

understood. Recent studies from our group have demonstrated that neurons activate GS as a protective response under physiological stress and that GS is required for enhancing the clearance pathways in the neurons. We extended our studies to find a direct link between GS and autophagy, and for testing the same, we have used neuroblastoma and primary neuronal cultures. Similar to the neuroblastoma lines, immunocytochemistry and biochemical analysis of the primary hippocampal neurons cultured at post-natal day 0 from wild type C57BL6/J mice revealed synthesis of glycogen when exposed to oxidative stress, with an increase in autophagy. Intriguingly, GS co-localized with LC3-positive autophagy vesicles upon exposure of neurons to physiological stress suggesting that overexpression of GS induced autophagy in the neurons. Intriguingly, real-time PCR (n=3) for GS overexpression in neuroblastoma led to the upregulation a few autophagy genes and conversely, knockdown of GS led their transcriptional downregulation. Thus, GS appears to regulate autophagy in neurons. In this paper, we will present our findings on the role of GS in healthy neurons and that undergoing neurodegeneration, making glycogen and its synthetic machinery as a potential therapeutic target for several brain disorders.

Disclosures: **A. Onkar:** None. **S. Ganesh:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Kanpur.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

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Program #/Poster #: 046.10/E5

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH T32 AG052375
P20GM103434
U54GM104942
P20GM103434

Title: Induction of cerebral hyperexcitability by peripheral viral challenge is mediated by CXCL10

Authors: ***T. J. PETRISKO**[1], J. BLOEMER[2], S. SRINIVASA[2], P. D. PINKY[2], R. T. HESLIN[2], Y. DU[2], S. E. SETTI[2], H. HONG[2], V. D. SUPPIRAMANIAM[2], M. REED[2], G. W. KONAT[1];
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Abstract: Peripheral viral infections are potent comorbid factors that exacerbate neurodegeneration; however, the underlying mechanisms have not been defined. In a quest to elucidate these mechanisms, we have developed a preclinical model, in which a viral mimetic, polyinosinic-

polycytidylic acid (PIC) is injected intraperitoneally to simulate peripherally-restricted viral challenge. We have demonstrated that PIC challenge elicits robust hyperexcitability of cerebral networks as seen from the development of seizure hypersusceptibility, increased basal synaptic transmission (BST), and the enhancement of long-term potentiation (LTP). Because neuronal hyperexcitability is a causative factor in neurodegeneration, our finding buttresses the contention that the enhancement of neuronal hyperexcitability is the putative mechanistic link between peripheral viral infections and exacerbations of neurodegeneration. At the molecular level, PIC challenge-induced hyperexcitability is concurrent with robust generation of cerebral CXCL10, a chemokine known to modulate neuronal activity. The present study was undertaken to determine the involvement of CXCL10 and its cognate receptor, CXCR3 in the development of neuronal hyperexcitability. Briefly, 8-week old female C57BL/6 mice were ip injected with 12 mg/kg PIC or equivolume saline, and after 24 h, the brains were analyzed. Confocal microscopy revealed CXCL10 to be generated primarily by neurons and astrocytes in the hippocampus and cortex. No CXCL10 generation was found in microglia. The expression of CXCR3 was confined to neurons. Blockage of CXCR3 through intracerebroventricular injection of an inhibitor, AMG-487 (3 mg/kg), abolished PIC-induced increase of BST and LTP. Based on these results, we posit that the activation of neuronal CXCL10/CXCR3 axis drives the development of hyperexcitability instigated by PIC challenge.

Disclosures: **T.J. Petrisko:** None. **J. Bloemer:** None. **S. Srinivasa:** None. **Y. Du:** None. **V.D. Suppiramaniam:** None. **M. Reed:** None. **G.W. Konat:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.11/E6

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIMH R01MH109382
NIH T32GM008275

Title: Mechanisms of PERK haplotype activity differences

Authors: *S. BOND[1], C. AKAY-ESPINOZA[3], K. L. JORDAN-SCIUTTO[2];
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[3]Pathology, Univ. of Pennslyvania Sch. of Dent. Med., Philadelphia, PA

Abstract: The Unfolded Protein Response (UPR) is a signaling system which aims to re-establish protein homeostasis under conditions of ER stress. It does so by sensing misfolding events in the ER with three master regulators, IRE1, ATF6, and PERK, which then affect signaling to increase the cell's folding capacity and maintain survival. Several markers of UPR

activation have been observed in the CNS of patients with HIV-Associated Neurocognitive Disorders (HAND). Indeed signs of UPR induction are observed across many age-related neurodegenerative diseases. Besides the adaptive function of the UPR, there is extensive evidence to suggest that severe or pro-longed stress can cause the cell to switch over to a mal-adaptive response, which could contribute to neurodegenerative phenotypes. The PERK arm of signaling has the most well established connections to such mal-adaptive responses, including prolonged translational attenuation leading to synaptic defects and induction of CHOP, a pro-apoptotic factor. PERK has two major haplotypes, A and B, differentiated by three single nucleotide polymorphisms (SNPs), which encode amino acid changes in the resulting protein. Intriguingly, haplotype B is a risk factor for several neurodegenerative diseases. Furthermore, haplotype B has been demonstrated to have increased kinase activity, as measured by phosphorylation of its canonical substrate, eIF2α, compared with haplotype A, in lymphocytes from homozygous donors, when subjected to the same endoplasmic reticulum stress. We thus hypothesize that the amino acid changes between PERK haplotypes cause haplotype B to respond more severely than A to the same ER stress in neurons, biasing it towards mal-adaptive signaling and disrupting normal neuronal function. Here we attempt to analyze the role of the individual changes in modulating overall PERK activity. Further studies will be needed to validate these results as well as the contributions of other mechanisms to haplotype B's enhanced activity. These studies may inform the development of novel therapeutics targeting PERK modulation for HAND and other neurodegenerative disease patients.

Disclosures: S. Bond: None. **C. Akay-Espinoza:** None. **K.L. Jordan-Sciutto:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.12/E7

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Modeling motor neuron diseases with iPSC-derived neurons

Authors: *R. PRICE[1], J. PINEDA[1], N. LIN[2], T. SMITH[2], A. W. ESSEX[1], J. EVANS[1]; [1]Phenovista Biosci., San Diego, CA; [2]Ixcells Biotechnologies, San Diego, CA

Abstract: Motor neuron diseases, including Amyotrophic Lateral Sclerosis (ALS), are a group of neurodegenerative disorders affecting motor neurons that have long suffered from a lack of quality *in vitro* models. We have developed a robust and scalable method to produce highly pure, functionally-validated, and ready-to-use motor neurons from human induced pluripotent stem cells (iPSC). These neurons show greater than 85% purity as measured by immunostaining of HB9, ChAT and Tuj1. These cells can be cryopreserved, thawed, and cultured in defined maintenance media with or without astrocytes, microglia or skeletal muscle for prolonged

culture. We have developed both patient-derived and isogenic models of ALS including lines carrying multiple mutations in the TDP-43 and SOD-1 genes. We have used high content imaging to provide phenotypic characterization of these models including differential expression and localization of various markers, including a re-distribution of TDP-43 protein from the nuclei to cytoplasm, differences in mitochondrial function, and responses to cellular stress (kainate and tunicamycin). These data show functionally relevant and measurable phenotypes in these human iPSC-derived neurons may be suitable for modeling MN diseases and in drug-screening assays.

Disclosures: **R. Price:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **J. Pineda:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **N. Lin:** A. Employment/Salary (full or part-time):; iXCells. **T. Smith:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **A.W. Essex:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences. **J. Evans:** A. Employment/Salary (full or part-time):; PhenoVista Biosciences.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.13/E8

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: POSTDOCTORAL FELLOWSHIP CONACYT GRANT 41133 TO GCH

Title: Methanolic extract of Lupinus exaltatus Zucc induces the expression of the stress response protein HSP-16.2 in *C. elegans*

Authors: *G. CAMARGO HERNANDEZ[1], S. SANCHEZ ENRIQUEZ[1], M. MALDONADO RUBIO[2], D. MENDOZA ARANDA[1], O. MARTINEZ ALVAREZ[1], M. GALLEGOS SAUCEDO[3], A. HERNANDEZ CHAVEZ[3], R. RODRIGUEZ MACIAS[4], J. BAÑUELOS PINEDA[4], L. HERNANDEZ[3];
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Abstract: INTRODUCTION: This is the first time that this wild species of *Lupinus* native from Mexico is tested in the model organism *C. elegans.* Oxidative stress is associated with aging, functional deterioration and increased mortality. This has supported studies on oxidative damage in animal models such as the nematode *Caenorhabditis elegans* (*C. elegans*). There are natural products with presumed antioxidant properties capable of reducing oxidative damage, such as the

methanolic extract of *Lupinus exaltatus Zucc* (*MELEZ*) an endemic plant of the states of the Central-western in Mexico. **AIM:** To evaluate the effect of *MELEZ*, against oxidative damage induced on the model organism *C. elegans*. **MATERIAL AND METHODS:** We used adult *C. elegans* N2 Wild type strain, and the transgenic strain TJ375. In N2 nematodes, survival at different doses of *MELEZ* was measured, and the effect of exposure to heat shock (HS) at 34°C for 1 h on the expression of HSP-16.2 protein was examined in strain TJ375:: GFP in the groups: control, HS, *MELEZ* and HS+*MELEZ*. **RESULTS:** *MELEZ* had no effect on the survival of the exposed nematodes. In nematodes of strain TJ375 exposed to HS an increase in the expression of HSP-16.2 was observed in relation to the control group. While treatment with *MELEZ* (0.5 mg/mL) during HS decreased the expression of HSP-16.2::GFP with respect to the control. However, without HS we observed an increase in the expression of HSP-16.2::GFP protein. **PRELIMINARY CONCLUSIONS:** Our results suggest that probably *MELEZ* induces protection by increasing the expression of proteins in response to stress. Furthermore, the ability of *MELEZ* could modulate the expression of some key genes in the insulin/IGF-1 signaling pathway (IIS), involved in longevity and oxidative or heat shock stress response.

Disclosures: G. Camargo Hernandez: None. **S. Sanchez Enriquez:** None. **M. Maldonado Rubio:** None. **D. Mendoza Aranda:** None. **O. Martinez Alvarez:** None. **M. Gallegos Saucedo:** None. **A. Hernandez Chavez:** None. **R. Rodriguez Macias:** None. **J. Bañuelos Pineda:** None. **L. Hernandez:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.14/E9

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Science and Engineering Research Board; Grant Number: PDF/2017/002471

Title: Neuromodulatory effects of excess glucocorticoid exposure: Studies on fly and mouse models

Authors: *P. MISRA[1], S. GANESH[2];
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Abstract: During most of the pregnancy, the fetal circulating levels of glucocorticoids are lower than the maternal levels because of the activity of the placental 11 β-hydroxysteroid dehydrogenase (HSD), which metabolizes most maternal cortisol to inactive metabolites. High level of maternal psychological/physical stress or pathological conditions impairing placental functions might lead to fetal exposure to excess glucocorticoids, exceeding the limit of placental

HSD. Emerging studies indicate that excessive prenatal exposure to glucocorticoid may have harmful effects on the developing fetus. One of such possibilities could be its detrimental effect on the neuronal cell survival pathways. Here, we used a synthetic analogue of glucocorticoid (GA) and tested its effect on the neuronal physiology using a cellular (Neuro2A, a neuroblastoma cell line) and a fly (*Drosophila*) model. We found that a concentration higher than 1 mM of GA significantly affected the survival of Neuro2A cells. Intriguingly, while a lower dose of GA decreased the levels of LC3-II and p62, indicating an increased autophagic flux, a higher dose increased the levels of LC3-II and p62, suggesting a compromised autophagy flux. The observed effect on autophagy was further tested using the cells ability to clear the mutant huntingtin protein; while the lower concentration of GA enhanced the clearance of mutant huntingtin, a higher level of GA led to the aggregation of the mutant protein and increased toxicity. Similar observations were made with the fly model expressing the mutant huntingtin. The observed effect of GA appears to be specific to neurons since a similar treatment did not affect the autophagy flux in COS7 cells. Taken together, our results suggest that a higher concentration of placental glucocorticoid might affect the neuronal physiology of the developing fetus and might serve as a risk factor for neurological disorders.

Disclosures: P. Misra: None. **S. Ganesh:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.15/E10

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH-RCMI-8G12MD007600

Title: Determining the inflammatory signaling pathways involved in cembranoid treatments on peripheral immune cells

Authors: *A. H. MARTINS[1], M. N. GONZALEZ-VEGA[2], **V. A. ETEROVIC**[2], A. ROCHE-LIMA[1], K. CARRASQUILLO-CARRION[1], P. A. FERCHMIN[2];
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Abstract: In neurodegenerative diseases, unregulated neuroinflammation affects neuronal integrity and increases tissue damage. When chronic inflammation is present in the central nervous system, peripheral immune cells accumulate in the brain parenchyma, passing through the blood-brain barrier (BBB); they are attracted by the signaling of activated microglia and macrophages recruited into the brain in a positive feedback fashion. Targeting prolonged inflammation in diseases like stroke, Parkinson's and Alzheimer's, is likely to provide more effective therapeutic measures than those presently available for these ills. (1S,2E,4R,6R,-

7E,11E)-2,7,11-cembratriene-4,6-diol (**4R**) is a cyclic diterpenoid that displays anti-inflammatory and neuroprotective activities in a series of ailments in rats. Those observations provided the rationale for the present study. The hypothesis of the present study is that **4R** modulates inflammation through NF-*κ*;B signaling pathway**.** To examine this premise through microarray analysis, macrophage cells (line Raw264.7) were treated with 4R after exposure to lipopolysaccharide (LPS). This study focused on two nuclear genes, PTGS2 and NF-*κ*;B1. When compared to the vehicle-treated group, 4R modulated both genes and affected ILK, PI3K/AKT, IL-17, PPAR, glucocorticoid-receptor signaling, and LXR/RXR activation (N=3 replications from 3 biological samples; $p<0.05$, p value adjusted Benjamini-Hochberg). At an RNA level, this exploratory study elucidated cell-signaling pathways activated by 4R to modulate inflammation and provided a valuable insight into the anti-inflammatory role of 4R. The results are likely to facilitate the development of treatments for the unregulated inflammatory insult caused by neurodegenerative diseases.

Disclosures: **A.H. Martins:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **M.N. Gonzalez-Vega:** None. **V.A. Eterovic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **P.A. Ferchmin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **A. Roche-Lima:** None. **K. Carrasquillo-Carrion:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.16/E11

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

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NIH: NIDDK(R01 DK104998)

Title: Knockout of the neuropsychiatric risk gene, Cacna1c, increases newborn neuron vulnerability through neuroinflammation

Authors: *M. F. NOTERMAN[1], M.-K. SHIN[2], E. VAZQUEZ-ROSA[2], C. CINTRÓN-PÉREZ[2], A. M. RAJADHYAKSHA[3], E. B. TAYLOR[1], A. A. PIEPER[4];

[1]Univ. of Iowa, Iowa City, IA; [2]Case Western Reserve Univ., Cleveland, OH; [3]Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY; [4]Psychiatry, Univ. Hosp., Cleveland, OH

Abstract: Neurons meticulously regulate intracellular calcium to permit its essential second messenger activity while minimizing its potential for excitotoxic calcium overload. L-type calcium channels (LTCC) allow activity-dependent calcium influx into neurons. This activity facilitates excitation-transcription coupling for long term potentiation. The seminal role of the predominant brain LTCC functional subunit, encoded by *CACNA1C*, is illustrated by its prominent implication in numerous neuropsychiatric diseases. However, the nature of the relationship between *CACNA1C* and etiologically-diverse neuropsychiatric disorders remains unknown. We previously showed that a transgenic mouse model with neuron-specific knockout (KO) of *Cacna1c* impairs survival of newborn hippocampal neurons, a cell population that is also negatively impacted in psychiatric diseases such as depression. Here, we investigated how *Cacna1c* regulates neuron viability. Using a global brain-*Cacna1c* KO mouse, we discovered that loss of *Cacna1c* increases inflammatory and oxidative brain damage. Furthermore, metabolomic analysis of global brain *Cacna1c* KO tissue revealed a basal hypoxic stress signature. To test the contribution of these stressors to neuron health, we measured the susceptibility of newborn adult hippocampal neurons to a traumatic brain injury. We found that brain-*Cacna1c* KO mice had decreased basal newborn neuron survival that was potentiated in severity by excitotoxic traumatic brain injury. Together, these data indicate that dysfunctional *Cacna1c* leads to cell stress that both basally and in injury states compromises cell viability. This poise towards neuron stress may make people with disease-implicated *CACNA1C* polymorphisms more vulnerable to developing neuropsychiatric diseases.

Disclosures: M.F. Noterman: None. **M. Shin:** None. **E. Vazquez-Rosa:** None. **C. Cintrón-Pérez:** None. **A.M. Rajadhyaksha:** None. **E.B. Taylor:** None. **A.A. Pieper:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.17/E12

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: SERB, (SR/SO/AS-027/2012, TCN)
Council of scientific and industrial research Senior research Fellowship

Title: Involvement of iron regulatory proteins in bright light induced stress in post hatch chick retina

Authors: *M. MAURYA[1], T. C. NAG[2], P. KUMAR[2], T. S. ROY[2], R. DADA[2];
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Abstract: The importance of iron in nutrition and cellular metabolism has been recognized for over a century. However, excess of cellular iron causes generation of reactive oxygen species (ROS) that play a detrimental role in various diseases. It is known that dysregulation of iron regulatory proteins such as transferrin (Tf), transferrin receptor-1 (TfR-1) and ceruloplasmin (Cp) results in altered iron homeostasis, leading to pathophysiology. On the other hand, their neuroprotective role has been emerged from many recent studies. For example, exposure to intense visible light is coupled with increased expression of ceruloplasmin (Cp) in retinal degeneration model. Since the role of iron transporters in light-induced retinal damage is unknown, the aim of this study is to understand if bright light causes oxidative stress and if so, how iron transporters are involved in this process.
One day-old chicks (*Gallus gallus domesticus*) were reared in normal 12 h light -12 h dark cycle (12L: 12D) for 7 days (400 lux). From day 8 (0h) onwards, they were exposed to 5000 lux (experimental) and 400 lux (control) light intensity and sacrificed at 168h. Cryosections were immunolabeled with Tf, TfR-1 and Cp antibodies, followed by immunoblotting. To evaluate retinal degeneration, transmission electron microscopy (TEM), TUNEL assay and Thiobarbituric acid reactive substances (TBARS) assay was done.
In 5000 lux exposed group, TEM analysis revealed degenerated outer segments, disorganized mitochondrial cristae in photoreceptor cells, organelle depletion in INL cells, gliosis of Müller cell processes and vacuolated axons in inner plexiform layers. An increase in lipid peroxidation level was seen in TBARS assay. Immunohistochemical localization of Tf was found to be intense in photoreceptors, inner nuclear layer (INL), ganglion cell layer (GCL), and in Müller cell processes. TfR-1 was extensively expressed in outer and inner plexiform layer and INL. Western blot analysis revealed increased expression of Tf ($p=0.03$) and TfR-1($p=0.04$), whereas Cp expression was decreased ($p=0.03$) in 5000 lux exposed groups.
Increased expressions of iron transporters Tf and TfR-1 implicate that retinal iron regulation is perturbed by bright light, while a downregulation of Cp supposedly associated with increase in free iron moiety, generating ROS. An increase in lipid peroxidation level and ultrastructural changes also supports these results. Such alterations implicate the adverse, secondary effect of light after photoreceptor damage and pivotal role of iron regulatory proteins in the protection of the retina from light-induced degeneration, most likely mediated via altered retinal iron levels.

Disclosures: M. Maurya: None. **T.C. Nag:** None. **P. Kumar:** None. **T.S. Roy:** None. **R. Dada:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.18/E13

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: National Health Research Institutes, Taiwan
Intramural Research Programs of the National Institute on Aging
National Institute on Drug Abuse, NIH

Title: Pifithrin-alpha reduces methamphetamine neurotoxicity in cultured dopaminergic neurons

Authors: Y.-H. CHEN[1], S.-J. YU[2], B. K. HARVEY[3], N. H. GREIG[4], ***Y. WANG**[2];
[1]Fu-Jen Catholic Univ., New Taipei City, Taiwan; [2]Natl. Hlth. Res. Inst., Zhunan, Taiwan; [3]NPR Section, NIDA - NIH, Baltimore, MD; [4]Drug Design & Develop. Section, LNS, Intramural Res. Program, Natl. Inst. On Aging, NIH, Baltimore, MD

Abstract: Methamphetamine (Meth) is a widely abused stimulant. High dose Meth induces degeneration of dopaminergic neurons through p53 -mediated apoptosis. A recent study indicated that treatment with the p53 inhibitor, pifithrin-alpha (PFT-α), antagonized Meth -mediated behavioral deficits in mice. The mechanisms underpinning the protective action of PFT-α against Meth have not been identified and hence their investigation is the focus of this study. Primary dopaminergic neuronal cultures were prepared from rat embryonic ventral mesencephalic tissue. High dose Meth challenge reduced tyrosine hydroxylase immunoreactivity and increased TUNEL labeling. PFT-α significantly antagonized these responses. PFT-α also reduced Meth -activated translocation of p53 to the nucleus, an initial step before transcription. Previous studies have indicated that p53 can also activate cell death through transcription-independent pathways. We found that PFT-α attenuated endoplasmic reticulum (ER) stressor thapsigargin (Tg) -mediated loss of dopaminergic neurons. ER stress was further monitored through the release of Gaussia luciferase (GLuc) from SH-SY5Y cells overexpressing GLuc-based Secreted ER Calcium -Modulated Protein (GLuc-SERCaMP). Meth or Tg significantly increased GLuc release in to the media, with PFT-α significantly reducing GLuc release. Additionally, PFT-α significantly attenuated Meth-induced CHOP expression. In conclusion, our data indicate that PFT-α is neuroprotective against Meth-mediated neurodegeneration via transcription-dependent nuclear and -independent cytosolic ER stress pathways.

Disclosures: Y. Chen: None. **S. Yu:** None. **B.K. Harvey:** None. **N.H. Greig:** None. **Y. Wang:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.19/E14

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant NS041234
NIH Grant NS101778
NIH Grant NS109252
NIH Grant NS076054

Title: Lysosomal regulation of inter mitochondrial contact fate and motility in charcot marie tooth type 2

Authors: *Y. C. WONG, W. PENG, D. KRAINC;
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Abstract: Properly regulated mitochondrial networks are essential for cellular function and implicated in multiple neurodegenerative diseases including Parkinson's and Charcot-Marie-Tooth disease. Mitochondria undergo fission and fusion events, but the dynamics, role and regulation of a third event of inter-mitochondrial contact tethering remain unclear. Using super-resolution imaging, we demonstrate that inter-mitochondrial contacts play a fundamental role in mitochondrial networks and functionally restrict mitochondrial motility. We recently found that mitochondria-lysosome contact site untethering is mediated by Rab7 GTP hydrolysis driven by its mitochondrial GAP (TBC1D15) and promotes mitochondrial fission. Here, we find that mitochondria-lysosome contact sites additionally promote inter-mitochondrial contact untethering events, which are further regulated by Mfn1/2 and Drp1 GTP hydrolysis to respectively promote inter-mitochondrial contact formation and untethering. Inter-mitochondrial contact untethering events are also marked by ER tubules which additionally mark mitochondrial fission and fusion events. Importantly, we find that multiple Charcot-Marie-Tooth Type 2 disease-linked mutations in Mfn2 (CMT2A), Rab7 (CMT2B) and TRPV4 (CMT2C) result in prolonged inter-mitochondrial contact tethering and disrupted mitochondrial motility, further highlight this pathway as a key contributor to mitochondrial homeostasis and a potential converging mechanism in peripheral neuropathy.

Disclosures: Y.C. Wong: None. **W. Peng:** None. **D. Krainc:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.20/E15

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Telfairia occidentalis mitigates the damaging effect of aluminium on the hippocampus of Wistar rats

Authors: A. VICTOR;
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Abstract: Aluminium is an environmental toxicant which accumulates in several organs of the body including the nervous system and damages them. The main mechanism of action is by inducing the excessive production of reactive oxygen species leading to oxidative stress. *Telfairia occidentalis* has been reported to possess an antioxidant, anti-inflammatory and immune modulatory properties and is investigated for its ameliorative effect on aluminium toxicity. Twenty adult male Wistar rats, weighing 120-150g were used and divided into four groups of five rats each. Group A (control) was administered 1ml of distilled water while each rat in every other group was administered 34mg/mkg of aluminium chloride. Group B was left untreated (toxic group), groups C and D were pre-treated with 100 and 400 mg/kg *Telfairia occidentalis* respectively approximately six hours before the administration of aluminium chloride. All administration was done orally with the aid of an oral cannula and it lasted for 7 weeks.
Results showed a significant depletion ($f = 6.009$; $p = 0.0061$) of the antioxidant system (SOD) of rats in groups B and C when compared with the control, except group D with no significant difference when compared with A. There was a significant suppression ($F = 12.46$; $p = 0.0002$) of glutamine synthetase enzyme activity of rats in group B when compared with groups A, C and D. A significant increase ($F = 7.217$; $p = 0.0028$) in hippocampal nitric oxide level of rats in groups B and C was recorded as against that of A. group D only shows no significant difference when compared with A. There was a significant increase ($F = 5.397$; $p = 0.0093$) in the level of aluminium ions found in the hippocampus of rats in groups B and C when compared with A. The current study revealed a reversal of the damaging effect of aluminium especially at 400mg/kg of the extract. The extract was believed to have a protective effect on the cells of the hippocampus which may be traceable to the presence of some phytochemical present in the extract. In conclusion, *Telfairia occidentalis* mitigates the toxic effect of aluminium chloride on the hippocampus.

Disclosures: A. Victor: None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.21/E16

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Decit/SCTIE/MoH
CNPq
CAPES
FAPERJ
FINEP
BNDES

Title: Role of copper imbalance in the infection of human induced pluripotent stem cells-derived astrocytes with zika virus

Authors: *T. PUIG-PIJUAN[1,2], L. R. Q. SOUZA[2], C. PEDROSA[2], R. H. F. VALVERDE[1], M. EINICKER-LAMAS[1], S. K. REHEN[2,3];
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Abstract: The zika virus (ZIKV) outbreak was responsible for an increase in congenital microcephaly cases and neurodevelopmental disorders. ZIKV infection caused oxidative stress, impaired cell proliferation and cell death in both neural progenitors and glia. Disturbances in copper levels are related to excessive production of reactive oxygen species (ROS) in the brain. Copper is an essential element for normal brain function, since it acts as a cofactor of enzymes like superoxide dismutase (SOD1) and cytochrome c oxidase. Copper imbalance is also linked to neurological diseases including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis. Besides, copper is essential in specific signaling pathways involved in cell proliferation, differentiation and apoptosis. Here, we tested the hypothesis of the involvement of copper imbalance in the deleterious effects caused by ZIKV in human neural cells.
In the brain, astrocytes are key regulators of both copper and redox homeostasis, which means their dysfunction can result in severe consequences for the developing central nervous system. For this reason, astrocytes were used as our model of study. Astrocytes derived from human induced pluripotent stem cells were incubated with ZIKV (MOI=1) for 2h. In order to remove available copper from cells, copper chelator Bathocuproinedisulfonic acid (BCS) was added 24h before or immediately after virus incubation. BCS did not change the percentage of ZIKV infected cells after 72 hours. However, BCS after ZIKV exposure significantly improved cell survival of infected astrocytes. Then, we analyzed the effect of BCS in ROS production. Copper

chelation either before or after ZIKV infection significantly reduced mitochondrial ROS levels and slightly reduced cytoplasmic ROS 48h after infection. Analysis of gene expression of copper-related proteins 72h after ZIKV-infection showed a twofold increase in the expression of Copper Chaperone for Superoxide Dismutase (CCS), which delivers copper to SOD1.
Our results suggest that copper is involved in the deleterious consequences of ZIKV infection, including oxidative stress and mitochondrial dysfunction, without affecting ZIKV infection rate itself. Alterations in SOD1 and other redox enzymes might also be implicated in the increase of ROS. Further research is necessary to further understand the specific pathways in which copper is involved.

Disclosures: **T. Puig-Pijuan:** None. **L.R.Q. Souza:** None. **C. Pedrosa:** None. **R.H.F. Valverde:** None. **M. Einicker-Lamas:** None. **S.K. Rehen:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.22/E17

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Neuroprotective effect of gintonin, a ginseng-derived ingredient, against 3-nitropropionic acid-induced Huntington's disease-like behavioral, biochemical, and cellular alterations

Authors: *Y. CHANG, M. JANG, J. CHOI, I.-H. CHO;
Kyung Hee Univ., Seoul, Korea, Republic of

Abstract: Gintonin (GT), a ginseng-derived lysophosphatidic acid receptor ligand, regulates various cellular effects and represses inflammation. However, little is known about the potential value of GT regarding inflammation in the neurodegenerative diseases, such as Huntington’s disease (HD). In this study, we investigated whether GT could ameliorate the neurological impairment and striatal toxicity in cellular or animal model of HD. Pretreatment with GT attenuated the severity of neurological impairment, lethality, mitochondrial dysfunction, apoptosis, microglial activation, and mRNA expression of inflammatory mediators in the striatum after 3-NPA-intoxication. Its action mechanism was associated with lysophosphatidic acid receptors (LPARs) and nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway activations and the inhibition of mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) signaling pathways. These beneficial effects of GT were neutralized by pre-inhibiting LPARs with Ki16425 (a LPAR1/3 antagonist). Taken together, our findings firstly suggested that GT has beneficial effects in 3-NPA-induced striatal toxicity by antioxidant and anti-inflammatory activities through LPA. Thus GT might be an innovative therapeutic candidate to treat HD-like syndromes.

Disclosures: **Y. Chang:** None. **M. Jang:** None. **J. Choi:** None. **I. Cho:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.23/E18

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: 1R21NS097899

Title: Role of mitophagy in hypoxia adaptation in Andeans

Authors: ***H. ZHAO**[1], G. G. HADDAD[2];
[1]Univ. of California San Diego, Sch. of Medicine, Dept. of Pediatrics, La Jolla, CA; [2]Dept. of Pediatrics, UCSD, La Jolla, CA

Abstract: Chronic mountain sickness (CMS) is a disease that potentially threatens a large segment of high-altitude populations (more than 100 million highlanders) that has been living at above 2500m for an extended period. Patients with CMS suffer from severe hypoxemia, excessive erythrocytosis and neurologic deficits including migraine headaches, tinnitus, mental confusion and cognitive deficits.The mechanisms underlying CMS neuropathology remain unknown. Our previous studies have shown that iPSC-derived CMS neurons have fragmentated mitochondria, decreased ATP level and increased susceptibility to cell death under hypoxia. Genome analysis from the same population has identified many mitochondrial, ER stress and Akt pathway genes that may play an important role in hypoxia adaptation.Therefore, we hypothesized that altered ER stress and PI3K/Akt signaling pathway may explain the difference observed between CMS neurons and those who do not show such CMS (non-CMS). In the current study, we first examined ER stress markers and found a significantly higher Grp78 expression in CMS neurons under normoxia and a significantly higher pPERK expression in CMS neurons after hypoxia, suggesting an increased ER stress under both normoxic and hypoxic conditions. ER stress is known to negatively regulate Akt pathway and, indeed, we observed a decreased pAkt/Akt in CMS neurons after hypoxic treatment for 24 hours. Since previous findings have demonstrated a link between Akt signaling and Pink and Parkin, two mitophagy genes that are central for mitochondrial quality control, we measured the expression of Parkin and LC3II, a mitophagy and autophagy marker respectively, in CMS and non-CMS neurons. Interestingly, we only found a decreased Parkin in CMS compared to non-CMS after hypoxia treatment for 24 hours, suggesting that 1) an compromised mitophagy in CMS neurons under stress as compared to non-CMS neurons, and 2) autophagy may not contribute to the difference observed between the two groups. Further studies are ongoing to investigate whether a compromised mitophagy plays an important role in the increased susceptibility to hypoxia observed in CMS neurons.

Disclosures: **H. Zhao:** None. **G.G. Haddad:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.24/E19

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: CCTS PECTS Program

Title: Extracellular vesicles as vehicles of toxicity in Krabbe disease

Authors: *C. R. REITER[1], J. MARSHALL[1], D. WOZNIAK[2], G. SCESA[1], D. NGUYEN[1], M. I. GIVOGRI[1], E. R. BONGARZONE[1];
[1]Univ. of Illinois at Chicago, Chicago, IL; [2]Aurora Univ., Aurora, IL

Abstract: Extracellular vesicles (EVs) are secreted by almost all mammalian cells to facilitate intercellular communication in various healthy and pathologic states. These vesicles contain various signaling molecules, such as protein and RNA, but they have also been shown to spread toxic accumulates such as α-synuclein (α-syn). Krabbe Disease (KD) is a devastating leukodystrophy caused by deficient lysosomal galactosylceramidase (GALC) catabolism of the prominent myelin sphingolipid, galactosylceramide and the toxic accumulation of a consequential byproduct galactosylsphingosine (psychosine). Psychosine buildup drives most of the observed pathology in KD, but the exact mechanism by which it induces its pathogenic effects remains unclear. Our team recently found psychosine to promote α-syn aggregation, and that the distribution and progression of α-syn deposits in KD follows the same Braak pattern observed in other synucleinopathies. Endogenous psychosine accumulates in lipid rafts and leads to altered membrane architecture, ultimately resulting in membrane shedding of vesicles. We hypothesize that these psychosine-containing vesicles propagate toxicity between different cell types in KD and that EVs may serve as a more generalizable mechanism of rostro-caudal patterns of toxicity in neurodegenerative disease. In this study, we have found elevated levels of psychosine and α-syn in EVs isolated from the brain of the Twitcher (Twi) mouse model of KD. These EVs transfer toxicity when added to naïve oligodendrocytes, microglia or astrocytes *in vitro*. To study the functional role of EV release *in vivo*, we utilized an inhibitor of neutral Sphingomyelinase 2 (GW4869) to deplete ceramide pools, which effectively decreases vesicle release. Twi mice treated with GW4869 had an earlier onset of disease phenotype, while wildtype mice were unaffected by treatment with vehicle or inhibitor. While overall brain psychosine levels were unchanged in all groups, treatment with GW4869 in Twi mice resulted in a significant decrease of brain-derived EVs and a consequential decrease in the levels of EV-associated psychosine compared to those treated with vehicle. Intriguingly, Twi mice treated with GW4869 exhibited region specific changes in myelination, as well as reactivity of both

astrocytes and microglia. These studies demonstrate that EVs have the potential to propagate toxicity and serve an important role in modulating the neuroinflammation found in KD and likely in other neurodegenerative conditions.

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Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.25/E20

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: 37(1)/14/27/2015/BRNS

Title: Benzo[a]pyrene-induced neuropeptide Y over expression and neurobehavioral changes in developing Wistar rats

Authors: *M. PATRI;
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Abstract: Benzo[a]pyrene (B[a]P) is a prototype of poly cyclic aromatic hydrocarbon (PAHs) known to induce neurobehavioral changes. Our previous reports address the adverse effect of B[a]P on the neurobehavioral responses and neuromorphology of sensitive brain regions in adolescent rats. Neuropeptide Y (NPY) is the most abundant peptide in the central and peripheral nervous system that involved in stress, memory, fear and anxiety Present study was conducted on male wistar rat neonates at postnatal day 5 (PND 5) to ascertain B[a]P-induced anxiolytic like behavioral response could be an outcome of NPY over expression in brain. Single intracisternal administration of B[a]P was carried out at PND 5 to elucidate the role of NPY on neurobehavioral responses at PND 35. The behavioral studies showed anxiolytic like effect of B[a]P in both light and dark box and elevated plus maze tests. Antioxidant assay involving glutathione peroxidase activity was significantly decreased where as lipid peroxidation was significantly augmented in both hippocampus and hypothalamus of B[a]P treated group as compared to naive and control. The neurotransmitter estimation by HPLC-ECD showed significant increase in serotonin (5-HT) level in both hippocampus and hypothalamus of B[a]P treated group. Significant elevation in NPY expression was observed in both hippocampus and hypothalamus of B[a]P group. Intracellular Ca^{2+} estimation using Fura-2AM by fluorimetry showed that B[a]P induced an increase in Ca^{2+} influx was associated with augmented NPY expression in brain. As NPY has orexigenic effect, our result revealed that there was a significant increase in body weight at PND 35 following B[a]P administration to rat neonates at PND 5. These findings suggested that NPY over expression in brain regions might be associated with

anxiolytic like behavioral response and orexigenic effect in rats following single intracisternal B[a]P administration. Future research directing towards understanding the signaling cascades of B[a]P induced biochemical and neuromorphological alteration might address the independent pathway which induce neurodegeneration despite NPY over expression in brain regions of adolescent rats.

Disclosures: M. Patri: None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.26/E21

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Genotype-phenotype relations of glycine decarboxylase and neuro-metabolic disease based on large-scale analysis of disease mutations

Authors: *J. FARRIS[1,2], S. ALAM[1,2], K. PAHAN[3], K. HALDAR[1,2];
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Abstract: Monogenetic diseases present privileged perspectives for studying complex molecular, cellular and organismal events that underlie neurological states. Loss of glycine decarboxylase (*GLDC*) can severely impact brain development resulting in neural tube defects (NTDs)[1,2] as well as the severe neuro-metabolic disorder non-ketotic hyperglycinemia (NKH)[3] often characterized by intractable seizures, failure to thrive, lack of developmental milestones, and premature death[4]. However, it is unknown how the 252 unique NKH-causing missense mutations that occur in GLDC affect P-protein's function and stability to impact disease severity. We describe large scale analyses and a novel model ranking method in protein-protein interaction predictions to relate all known NKH mutations to severity of neuro-metabolic disease. We predict new functions for the *GLDC* N-terminal domain in binding pyridoxal phosphate (PLP), a known GLDC co-factor, revealing a functional rationale for N-terminal NKH mutations. We also predict that mutations disrupting GLDC's association with a key interactor can cause neuro-metabolic disease. Ongoing analyses of these and additional findings have collectively yielded (i) new perception of GLDC-induced NKH disease and (ii) the first missense mouse model of severe NKH disease. This work provides important insights into understanding the molecular mechanisms by which metabolic factors control seizures and other neuro-cognitive defects in NKH as well as more prevalent neurological conditions.
1. Narisawa, A. *et al.* Mutations in genes encoding the glycine cleavage system predispose to neural tube defects in mice and humans. *Hum. Mol. Genet.* **21**, 1496-1503 (2012).
2. Pai, Y. J. *et al.* Glycine decarboxylase deficiency causes neural tube defects and features of

non-ketotic hyperglycinemia in mice. *Nat. Commun.* **6**, (2015).
3. von Wendt, L., Hirvasniemi, A. & Similä, S. Nonketotic hyperglycinemia. A genetic study of 13 Finnish families. *Clin. Genet.* **15**, 411-7 (1979).
4. Hennermann, J. B., Berger, J. M., Grieben, U., Scharer, G. H. & Van Hove, J. L. K. Prediction of long-term outcome in glycine encephalopathy: A clinical survey. *J. Inherit. Metab. Dis.* **35**, 253-261 (2012).

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Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.27/E22

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Alzheimer's Association Grant AARF-17-531426
NIH Grant AG008200

Title: PS1 FAD mutants attenuate trophic factor-dependent neuroprotection via altering NMDAR-trophic factor receptors interactions with PS1

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Abstract: Mutations in the *PSEN1* gene, encoding presenilin-1 (PS1), are the most common cause of familial Alzheimer's disease (FAD), which may share common pathogenic mechanisms with the more common sporadic Alzheimer's disease (AD). Increased vulnerability of the brain to toxic insults such as excitotoxicity, ischemia and oxidative stress, contributes to AD neurodegeneration. NMDA receptor (NMDAR), a major ionotropic glutamate receptor, modulates neuronal responses to toxic insults, and may interact with brain neurotrophins to protect neurons from excitotoxicity and oxidative stress. We have reported that PS1 promotes neuronal survival by regulating neuroprotective functions of BDNF and ephrinB (efnB), called here 'Factors'. Here, we found that PS1 was also required for the induction of NMDA-Factor receptor interactions which underlie neuroprotection. We also observed that PS1 FAD mutants (M146V, I213T) attenuated both, Factors-induced stimulation of NMDA-Factor receptor interactions, and neuroprotection. Furthermore, FAD mutants led to an abnormal accumulation of PS1 in the membrane synaptosomal fractions, thereby perturbing the stoichiometry of constitutive NMDA-Factor receptor interactions with PS1, resulting in diminished neuroprotective functions of Factors. More importantly, we found congruent data in human

postmortem brains carrying PS1 FAD mutation, providing further credence to our findings in the AD mechanism. Our data reveal a mechanism by which FAD mutants impart intrinsic neurodegenerative vulnerability to excitotoxicity by dominantly incapacitating Factors-dependent neuroprotection mechanism. Agents that target this novel neuroprotection mechanism may have therapeutic value in FAD.

Disclosures: **M.A. Rahim:** None. **Z. Shao:** None. **C. Dimovasili:** None. **A. Georgakopoulos:** None. **N. Robakis:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.28/E23

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH R01 DK116624
AHA predoctoral fellowship
NIH R21 MH113352

Title: Targeting mitochondrial fission for neuroprotection in peripheral diabetic neuropathy

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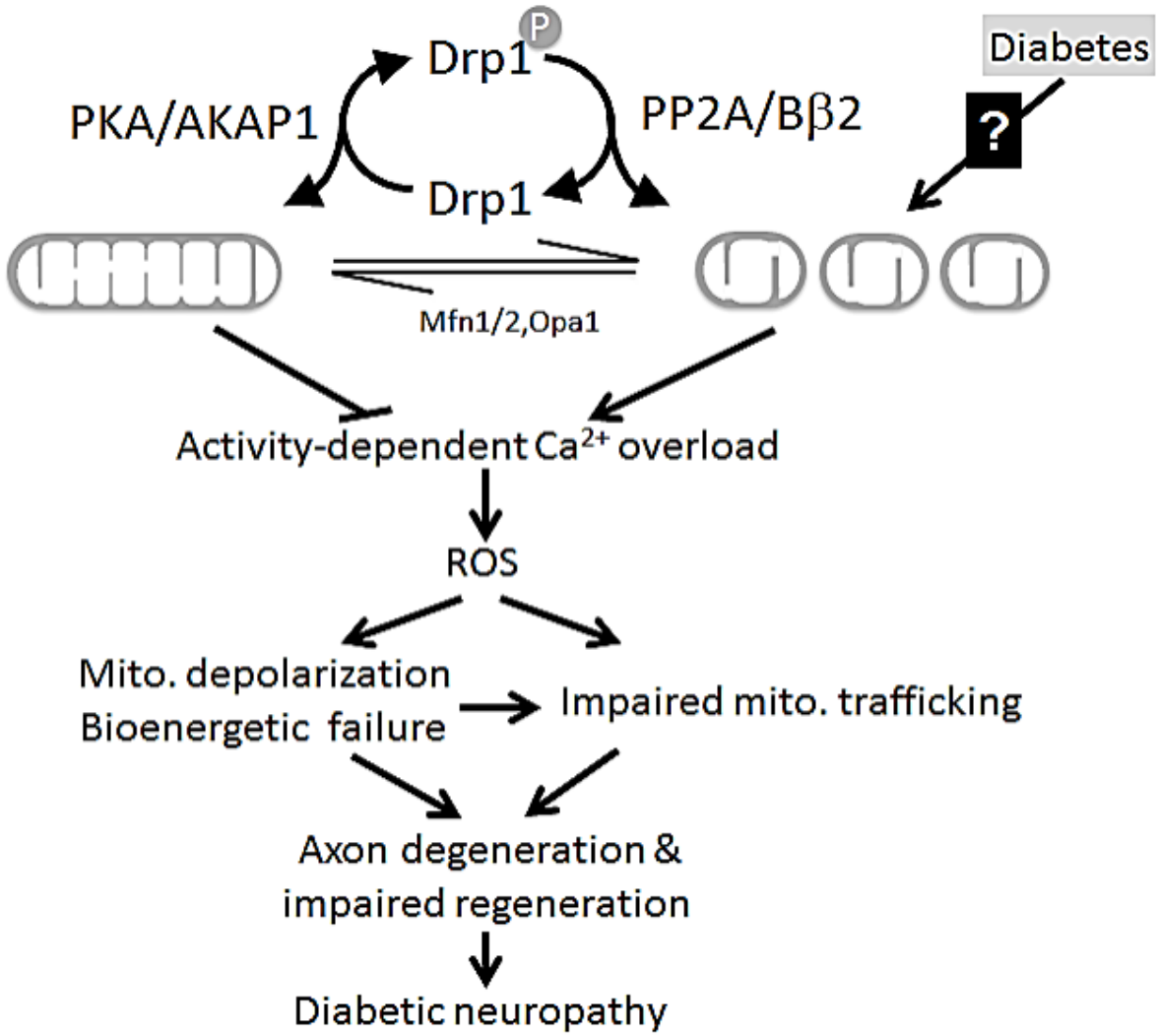
Abstract: Diabetes has become a fully-blown global epidemic requiring global attention. Presenting with loss of sensation and chronic pain, peripheral diabetic neuropathy (PDN) is a debilitating co-morbidity affecting at least 50% of the diabetic patients. With palliative care being the only option, there is an urgent need of innovative therapies for PDN. Emerging evidence recently indicated compromised mitochondria structure and function in diabetes. Mitochondria forms highly dynamic networks that constantly undergoing the process of fission and fusion, governed by the dynamin family of large GTPases. Balanced fission and fusion are required for proper function of mitochondria. Interestingly, excessive mitochondrial fission was implicated in many neurodegenerative disorders including Alzheimer's and Huntington's diseases. Therefore, we investigated whether targeting mitochondrial fission can be a potential therapeutic strategy for PDN. Dynamin-related protein 1 (Drp1) is an essential mitochondrial fission enzyme. Drp1 is activated by dephosphorylation via two phosphatases including calcineurin and a neuron-specific, mitochondria localized isoform of protein phosphatase 2A

containing the Bβ2 regulatory sub-unit (PP2A/Bβ2). We generated the Bβ2 knock-out (KO) mouse in which we observed elongated mitochondria in neurons as well as reduced high-glucose induced superoxide production in their DRG neurons. Bβ2 KO mice also showed increased mitochondrial axonal localization, while preventing axonal mitochondrial fission and depletion in sciatic nerve in the STZ model of type-1 diabetes. These animals were shown to be protected from thermal hypoalgesia. Moreover, KO of Bβ2 also showed protection from both thermal and mechanical hypoalgesia as well as impaired nerve conductivity in db/db mice modeling type-2 diabetes. Taken together, our preliminary data showed that Bβ2 KO animals are resistant to PDN in both type-1 and type-2 diabetes models. Hence, targeting mitochondrial fission through PP2A/Bβ2 shed light onto developing an innovative treatment for PDN.



Disclosures: **Y. Liu:** None. **K.H. Flippo:** None. **R.A. Merrill:** None. **A.S. Dickey:** None. **L. Shutov:** None. **M. Yorek:** None. **Y.M. Usachev:** None. **S. Strack:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.29/E24

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: CONACyT fellowship 373878 to MJGS

Title: Dehydroepiandrosterone sulfate lends protection against damage induced by chemical hypoxia in *Caenorhabditis elegans* GABAergic system

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Abstract: Hypoxia is involved in pathophysiological processes leading to several medical conditions, the nervous system is particularly affected. We postulate that the GABAergic system is especially sensitive. Drugs improving the resistance to hypoxia have been investigated, such as the neurosteroid Dehydroepiandrosterone Sulfate (DHEAS) which have shown beneficial effects in hypoxic processes in mammals, though, at the cellular level, its exact mechanism of action has yet to be fully elucidated. Here, we applied a chemical hypoxia model in *Caenorhabditis elegans* (*C. elegans*), a nematode whose response to hypoxia involves pathways and cellular processes conserved in mammals. The aim of this work was determining the effect of DHEAS on damage to the GABAergic system associated with hypoxia in *C. elegans*. We established an untreated group (CTL), a hypoxic group (HPX) and a hypoxic group with DHEAS (HPX+DHEAS). Worms were subjected to Nose touch response (Not Assay) and observed in Nomarski and epifluorescence microscopy. DHEAS decreased the severity of hypoxic injuries in the pharynx. Shrinkage response of Not Assay and the level of severe damage in GABAergic neurons were significantly less frequent in HPX+DHEAS group than HPX worms. Also, the enhanced nuclear localization of DAF-16 and consequently the overexpression of chaperone HSP-16.2 by hypoxia were significantly reduced in HPX+DHEAS worms. These results suggest that hypoxia-caused damage over the GABAergic system was prevented at least partially by DHEAS.

Disclosures: L. Hernandez: None. **M.J. Gallegos-Saucedo:** None. **A. Castillo-Romero:** None. **R. Cortéz-Zárate:** None. **A.L. Pereira Suárez:** None. **M.A. Ramirez-Herrera:** None. **J. Bañuelos-Pineda:** None. **A. Hernández-Chávez:** None. **G. Camargo:** None.

Poster

046. Cell Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 046.30/E25

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Peripheral and central nervous systems changes during primary varicella zoster virus infection in guinea pig

Authors: *C. S. NIEMEYER, T. MESCHER, C. N. COMO, J. E. ORFILA, A. N. BUBAK, J. BETKER, T. ANCHORDOQUY, M. A. NAGEL;
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Abstract: Varicella zoster virus (VZV) is a neurotropic alphaherpesvirus that produces varicella on primary infection (chicken pox), establishes latency in ganglionic neurons then reactivates to cause herpes zoster (shingles) with aging or immunosuppression. During primary infection and reactivation, VZV can also infect multiple organs to produce multisystem disease including stroke, cranial nerve palsies, vasculopathy, and encephalitis. A critical barrier in dissecting mechanisms of VZV-associated disease is the lack of a reproducible, well-characterized small animal model for VZV infection. Prior studies have demonstrated that the guinea pig (*Cavia porcellus*) is a candidate model with establishment of latency in cranial nerve, dorsal root and enteric ganglia after intravenous inoculation of VZV; reactivation occurs with tacrolimus and corticotrophin-releasing hormone. We reproduced and optimized these studies for a comprehensive characterization of primary VZV infection in the guinea pig. Outbred, IAF hairless guinea pigs with either jugular vein or common carotid artery catheters were used to facilitate inoculations and blood draws. VZV-infected peripheral blood mononuclear cells (PBMCs) were able to transmit infection when re-injected through the catheter into the same animal. A transient viremia was seen within 5 days post-infection (DPI). From 15-17 DPI, 3/3 VZV-infected guinea pigs developed clusters of raised, erythematous skin lesions, similar to human varicella rash, in cervical dermatomes. Preliminary hippocampal slice physiology experiments demonstrated robust long term potentiation providing a baseline to compare physiological changes induced by VZV infection. Our findings demonstrate a well-defined protocol for establishing a small animal model of VZV infection that holds promise for studying the pathogenesis of primary infection *in vivo*, as well as examining central nervous system changes associated with peripheral disease.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.01/E26

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Japanese Society for the Promotion of Science 24591360
Research on Hypothalamo-hypophyseal Disorders from the Ministry of Health, Labour andWelfare, Japan

Title: NMDA receptor antagonist prevents cell death in the hippocampal dentate gyrus induced by hyponatremia accompanying adrenal insufficiency in rats

Authors: *H. FUJISAWA[1], H. IZUMIDA[2], A. SUZUKI[1], Y. SUGIMURA[1];
[1]Dept. of Endocrinol. and Metabolism, Fujita Hlth. Univ., Toyoake, Japan; [2]Nishichita Gen. Hosp., Tokai, Japan

Abstract: Selective apoptosis of granule cells in the hippocampal dentate gyrus (DG) of rats with bilateral adrenalectomy (ADX) and in patients who died of adrenal insufficiency has been reported. Although adrenal insufficiency is a common disease and is usually associated with hyponatremia, its effect on the central nervous system and in apoptosis in the hippocampus remain to be elucidated. In order to evaluate the pathophysiology of hyponatremia accompanying adrenal insufficiency, we developed chronic hyponatremic rat models with adrenal insufficiency similar to clinical settings by ADX followed by feeding low sodium liquid diet. Almost all hyponatremic rats were alive on day 7. Subsequently, almost all rats with serum <125 mEq/L (moderate hyponatremia) died between days 9 and 11. Nine days after ADX, apoptotic cells were observed in the DG in moderate hyponatremic rats, but rarely in those whose serum [Na^+] was ≥125 mEq/L or in normonatremic rats. Apoptotic cells were not observed in other areas of hippocampus. Although all hyponatremic ADX rats survived following treatment with corticosterone (CORT) and saline started 7 days after ADX when apoptosis had not yet occurred, selective apoptosis on day 9 was not prevented in moderately hyponatremic rats treated with CORT. In addition, there were more doublecortin positive cells, activated microglia and enlarged astrocytes in the DG of these rats than that of naïve rats, suggesting that the pathophysiological stress or apoptosis associated with hyponatremia may enhance DG neurogenesis and that microglial activation. Interestingly, treatment with memantine, a noncompetitive NMDAR antagonist, prevented the selective apoptosis in the DG in moderately hyponatremic, ADX rats, and improved electrophysiological dysfunction, including impaired basal synaptic transmission and long-term potentiation at the entorhinal cortex-DG synapses. These results demonstrated that in adrenal insufficient rats, hyponatremia was associated with apoptosis in the DG, and that memantine, but not the conventional treatment with CORT replacement without memantine,

prevented the apoptosis and improved cell function. Our data imply the importance of assessing the possibility of neurological impairments after treatment with CORT in patients with moderate or severe hyponatremia accompanying adrenal insufficiency and that memantine may represent a beneficial therapeutic strategy to prevent neurological impairments in such patients.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.02/E27

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Intramural Research Programs of the National Eye Institute
The European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 749346

Title: Neuroprotection of retinal ganglion cells by bone marrow mesenchymal stem cells-derived small extracellular vesicles

Authors: *S. I. TOMAREV[1], Z. AHMED[2], B. MEAD[1];
[1]NEI, NIH, Bethesda, MD; [2]Inst. of Inflammation and Ageing, Col. of Med. and Dent. Sciences, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: We have previously demonstrated that small extracellular vesicles (SEV) derived from human bone marrow mesenchymal stem cells (BMSC) but not from human fibroblasts provide retinal ganglion cell (RGC) neuroprotection in rodent models of optic nerve crush (ONC) and glaucoma. SEV miRNAs play an essential role in the observed neuroprotection. Our current study has 3 aims: 1), characterize changes in the RGC miRNA spectrum in a rat laser glaucoma model and after ONC; 2), characterize changes in RGC mRNAs after intravitreal injection of SEV and; 3), assess whether the neuroprotective efficacy of SEV can be improved by priming BMSC prior to isolation. SEV were isolated from human BMSC and fibroblasts and characterized by NanoSight and Western blot. SEV were intravitreally injected at a concentration of $3x10^9$ weekly. RGCs were isolated from adult rat retina homogenates by immunopanning. RNAseq was used to quantify miRNAs present in BMSC- and fibroblast-derived SEV as well as purified RGCs with or without SEV treatment. BMSC were primed by incubation with TNFα for 48h before SEV isolation. ONC or elevation of intraocular pressure (IOP) led to statistically significant changes in the abundance of 127 RGC miRNAs compared with control non-damaged samples. These changes were minor 2 days after ONC but more pronounced 7 days after ONC or after IOP elevation. Moreover, changes induced by IOP elevation were quite different from changes induced by ONC. Several mRNA targets were identified for miRNAs with a modified

abundance. Preliminary data suggest that intravitreal injection of BMSC-derived SEV led to the modification of RGC gene expression pattern 7 days after ONC compared with ONC alone. Priming BMSC prior to isolation of SEV led to pronounced changes in SEV miRNA composition compared with unprimed SEV and improved their neuroprotective efficacy after ONC. These data indicate that changes in miRNA composition may play a role in RGC neuroprotection after ONC or IOP elevation and that different miRNAs may be essential for neuroprotection in different models of RGC degeneration.

Disclosures: S.I. Tomarev: None. **Z. Ahmed:** None. **B. Mead:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.03/E28

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant R01 NS076054
NIH Grant R37 NS096241

Title: Regulation of calcium dynamics at mitochondria-lysosome contact sites

Authors: *W. PENG, Y. C. WONG, D. KRAINC;
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Abstract: Mitochondria and lysosomes are both key organelles for regulating cellular calcium, an ion which is responsible for modulating multiple neuronal functions including neurotransmitter release, ATP production and neuronal excitability. Our lab recently identified novel inter-organelle membrane contact sites which form between mitochondria and lysosomes, whose untethering is regulated by Rab7 GTP hydrolysis, and is important for modulating mitochondrial dynamics. However, whether mitochondria-lysosome contact sites can further serve to regulate neuronal homeostasis by acting as platforms for regulating calcium transfer and homeostasis remains unknown. Using live cell imaging, we find that activation of lysosomal calcium efflux leads to increased mitochondrial calcium levels. We further show that this calcium transfer from lysosomes to mitochondria is further modulated by increasing mitochondria-lysosome contact tethering. Finally, we show that lysosomal calcium efflux modulates both mitochondrial and lysosomal dynamics and function. As loss of function mutations in several lysosomal calcium channels are linked to neurological disease, disruption of calcium dynamics at mitochondria-lysosome contact sites may play a role in disease pathogenesis.

Disclosures: W. Peng: None. **Y.C. Wong:** None. **D. Krainc:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.04/E29

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Investigating the role of serotonin receptor 2A inhibition in oligodendrocyte precursor maturation and *in vitro* myelination

Authors: *S. GIERA, K. RADZWILL, G. SHENG, J. E. FARLEY, C. GARRON, C. PEDRAZA;
Sanofi, Framingham, MA

Abstract: Multiple sclerosis (MS) pathogenesis leads to demyelinated axons causing dysfunction of propagated signals, axonal injury, neuronal loss, accumulation of lesion load and overt neurological disabilities. While endogenous remyelination by oligodendrocyte precursor cells (OPCs) repair and limit the damage in early stages of MS, during disease progression the repair process no longer occurs with the same efficiency. Remyelination can be stimulated by pharmacological means and it is the focus of active research in drug development for MS therapeutics. We have identified selective and potent serotonin receptor (5-hydroxytryptamine receptor 2A, HTR2A) antagonists, showing high efficacy (EC50 <1 microM) induction of oligodendrocyte precursor (OPC) maturation and in vitro myelination of artificial fibers. Additionally, we have observed increased expression of HTR2A in postmortem MS samples and in animal models of MS. Although HTR2A is primarily expressed in neurons and at lower levels in cells of the oligodendrocyte lineage, regulation of its activity has been previously suggested to have a significant impact on OPC maturation (Quetiapine, Paroxetine, Clemizole). HTR2A antagonists (inverse agonists) show high selectivity versus other neurotransmitter receptors, especially against other members of the serotonin receptor family, high brain penetration and proven HTR2A pharmacological actions in in vivo models. Further validation of HTR2A as a target for enhancement of myelin regeneration will enable testing the rationale in early proof of concept clinical trials for remyelination therapies.

Disclosures: **S. Giera:** A. Employment/Salary (full or part-time):; Sanofi. **K. Radzwill:** A. Employment/Salary (full or part-time):; Sanofi. **G. Sheng:** A. Employment/Salary (full or part-time):; Sanofi. **J.E. Farley:** A. Employment/Salary (full or part-time):; Sanofi. **C. Garron:** A. Employment/Salary (full or part-time):; Sanofi. **C. Pedraza:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.05/E30

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A1B07051112)

Title: Small leucine-rich proteoglycan: A novel pathogen candidate for Parkinson's disease

Authors: E. BOK, M. LEE, J. BAEK, ***W.-H. SHIN**;
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Abstract: Parkinson’s disease (PD) is a common neurodegenerative disorder whose pathogenesis remains unknown. A small leucine-rich repeat proteoglycan (SLRP) is an extracellular matrix component that exists in the brain. SLRP acts as an endogenous ligand of Toll-like receptors (TLR)-2/4 and involvement of SLRP in numerous diseases has been reported. However, little is known about the role of SLRP in brain disorders. In the present study, immunohistochemistry staining and western blot analysis showed that SLRP expression was increased in substantia nigra (SN) of postmortem PD brains. The intranigral injection of recombinant SLRP induced a loss of dopaminergic (DA) neurons and microglial activation in the rat SN *in vivo* as visualized by tyrosine hydroxylase (TH) and OX-42 immunohistochemistry staining, respectively. Interestingly, mesencephalic neurons were more vulnerable to SLRP-induced toxicity than cortical, hippocampal neurons and B103 neuroblastoma cells *in vitro*. These our results suggest that SLRP may be an endogenous pathogen for PD.

Disclosures: E. Bok: None. **M. Lee:** None. **J. Baek:** None. **W. Shin:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.06/E31

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: IMI2 Grant 821522

Title: The effects of local energetic stress on mitochondrial transport in primary cortical axons

Authors: *O. WATTERS[1], N. M. C. CONNOLLY[1,2], H.-G. KÖNIG[1], H. DÜSSMANN[1,2], J. H. M. PREHN[1,2];
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Abstract: Mitochondria play an important role in the maintenance of neuronal homeostasis by generating energy in the form of ATP. Axons rely heavily on lactate as a substrate for ATP synthesis, provided through the astrocyte-neuron lactate transfer shuttle. A change in cellular ADP / ATP ratio during energetic stress induces activation of the energy stress sensor, AMPK. AMPK signalling cascades work to restore energetic homeostasis in the cell. Effects of AMPK on mitochondrial transport dynamics along neuronal processes are less well understood. In this study, we investigated the effects of localised lactate deprivation and AMPK activation on mitochondrial transport in primary cortical axons. Primary cortical neurons were nucleofected with mito-GFP and cultured within microfluidic devices to create physical isolation of axonal processes from somato-dendritic regions. At 8 DIV, the movement of GFP-positive axonal mitochondria was monitored using time-lapse confocal microscopy. Kymographs were generated and analysed to assess changes in axonal mitochondria transport over time. Repeated measures ANOVA and Dunnett's test were used to compare the pooled data from each 30 min interval with their corresponding baseline values. A strict statistical significance threshold of p-values ≤ 0.01 was set to account for alterations due to high-frequency imaging acquisition (0.25 Hz). Direct activation of AMPK at the distal axon (AICAR, 0.1 mM) reduced the frequency, velocity and distance of mitochondrial transport in the adjacent axon, which was restored by pharmacological inhibition of AMPK (compound C, 10 μM). AICAR treatment at the somato-dendritic compartment failed to alter mitochondrial transport in the spatially isolated axon, confirming the regional-specific action of AMPK on mitochondrial transport at the distal axon. To investigate AMPK activity in a more physiological setting we exposed the distal axon to lactate and induced localised nutrient deprivation in this region by inhibition of lactate uptake (AR-C155858, 10 nM). Localised nutrient deprivation at the distal axon induced a depression in the frequency, velocity and distance of mitochondrial transport in the adjacent axon similar to that observed by localised AICAR treatment. Co-addition of compound C successfully restored all parameters measured to baseline levels, confirming the involvement of AMPK in localised changes in mitochondrial mobility during nutrient deprivation. This study highlights a role of the energetic stress signalling molecule AMPK, in the modulation of axonal mitochondrial mobility during localised nutrient deprivation.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.07/E32

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Modeling of Parkinson's disease (PD) related pathophysiology in primary human dopaminergic neurons: Role of autophagy and protein aggregation

Authors: *S. F. ALI[1], E. CUEVAS[2], A. GUZMAN-LOPEZ[3], H. ROSAHERNANDEZ[4], Z. ZHANG[6], S. M. LANTZ[7], S. IMAM[5];
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Abstract: The pathophysiology underlying the loss of dopaminergic (DA) neurons in Parkinson's disease (PD) is still unclear. A major barrier to the development of effective therapies for PD is the current limitation in our understanding of the molecular and cellular events that lead to degeneration of the nigrostriatal DA system. MPP^+ (1-methyl-4-phenylpyridinium) has been used as a reliable *in vitro* model of PD in dopaminergic neurons. However, the molecular mechanisms are not fully understood. Here, we characterized the expression of PD-related proteins and neuronal death after exposure of human DA neurons to MPP^+ at different concentrations (0-5 mM) and time points (4, 8 and 24 h). Viability assays (LDH, XTT, Mitotracker, or live and dead staining) as well as molecular markers (lysosome-associated membrane protein 1 (LAMP-1), light chain 3 (LC3), tyrosine hydroxylase (TH), α-synuclein (α-SYN) and parkin) were evaluated. MPP^+ significantly reduced the cell viability, decreased mitochondrial labeling, and reduced the number of live cells, only after 24 h. Molecular analyses were performed only at 1 and 2.5 mM MPP^+. Expression of the autophagy marker LAMP-1 was increased after MPP^+ treatment. The ratio of LC3B2/LC3B1 was also increased. Expression of TH was decreased. The localization of parkin and aggregation of α-SYN was cytoplasmic and nuclear, and α-SYN was more prominent in the nucleus. These data suggest that MPP^+ could be affecting pathways related to autophagy, dopamine synthesis and, protein aggregation, justifying its use as an *in vitro* model of PD, which may aid in the future research of this disease. (Supported by NCTR/FDA protocol E0761601)

Disclosures: S.F. Ali: None. **E. Cuevas:** None. **A. Guzman-Lopez:** None. **H. RosaHernandez:** None. **Z. Zhang:** None. **S.M. Lantz:** None. **S. Imam:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.08/E33

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Astrocyte-like cells derived from donors with globoid cell leukodystrophy display cell autonomous and non-cell autonomous effects associated with disease

Authors: R. LIEBERMAN[1], G. Y. GAO[1], H. PARK[2], R. H. BARKER, Jr[1], J. P. LEONARD[1], ***B. HUNTER**[1], L. K. CORTES[1];
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Abstract: Globoid cell leukodystrophy (Krabbe disease) is a fatal neurodegenerative, demyelinating disease caused by dysfunctional activity of galactosylceramidase (GALC), which leads to the accumulation of glycosphingolipids including psychosine. The cell types contributing to disease pathogenesis are continuing to be elucidated, but the identification of cell autonomous and non-cell autonomous effects linked to GALC dysfunction may facilitate a better understanding of cell types important to target with novel therapeutics. To this end, we generated induced pluripotent stem cells (iPSCs) from two donors with the severe infantile form of the disease and subsequently differentiated them into cultures of astrocyte-like cells. Compared to astrocytes from three healthy control donors, Krabbe astrocytes accumulated psychosine and had higher expression of the pro-inflammatory cytokine IL-6, recapitulating findings in humans and rodents. Interestingly, we identified that Krabbe astrocytes had higher levels of ceramide and glucosylceramide and displayed compensatory changes in genes encoding enzymes in the glycosphingolipid biosynthetic pathway, suggesting shunting away from the galactosylceramide arm. In co-culture systems, Krabbe astrocytes failed to support survival of neurons but supported survival of microglia-like cells. A substrate reduction approach targeting either serine palmitoyltransferase to reduce ceramide and its downstream metabolites or glucosylceramide synthase to reduce glucosylceramide failed to facilitate Krabbe astrocyte support of neurons and failed to reduce elevated expression of IL-6. Our results suggest that astrocytes may contribute to the progression of Krabbe disease and warrant further exploration into their role as therapeutic targets.

Disclosures: **R. Lieberman:** A. Employment/Salary (full or part-time):; Sanofi. **G.Y. Gao:** A. Employment/Salary (full or part-time):; sanofi. **H. Park:** A. Employment/Salary (full or part-time):; sanofi. **R.H. Barker:** A. Employment/Salary (full or part-time):; Sanofi. **J.P. Leonard:** A. Employment/Salary (full or part-time):; Sanofi. **B. Hunter:** A. Employment/Salary (full or part-time):; Sanofi. **L.K. Cortes:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.09/E34

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: 5R01EY024481-05 (PAR, LIB)
5R01EY027881-02 (PAR, LIB)
Adelson Medical Research Foundation (LB)

Title: Inhibition of synaptic zinc release by tetanus neurotoxin promotes retinal ganglion cell survival and axon regeneration following optic nerve injury

Authors: *E. G. SERGEEVA[1], Y. LI[3], L. I. BENOWITZ[2], P. A. ROSENBERG[1];
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Abstract: The death of the RGCs and their failure to regenerate axons after optic nerve injury leaves patients with glaucoma and nerve trauma irreversibly blind. In a mouse model, we previously demonstrated by autometallography (AMG) that one consequence of optic nerve crush (ONC) is the accumulation over the first 24 hours of mobile zinc in synaptic vesicles of amacrine cell (AC) terminals in the retinal inner plexiform layer (IPL). Removal of zinc with metal chelators promoted RGC survival and axon regeneration [Li et al., *PNAS* 2017]. Here, we tested the hypothesis that the negative effects of zinc on RGC survival and axon regeneration requires vesicular zinc to be released from AC terminals. We inhibited zinc release using *Clostridium tetani* neurotoxin (TeNT), which selectively targets inhibitory neurons and, via cleavage of a SNARE protein synaptobrevin, inhibits exocytosis of presynaptic vesicles. We injected TeNT (2, 20, 200 nM) into the eye immediately after ONC (129S1 male mice) and, two weeks later, harvested retinas and optic nerves, and immunostained RGCs for beta III tubulin and regenerating axons for GAP-43. In additional cohorts of mice, we used AMG to visualize accumulation of vesicular zinc. In intact mice, TeNT induced a dose-dependent increase in AMG staining in the IPL after 3 days. Mice lacking the vesicular zinc transporter ZnT3 did not respond to TeNT with increase in AMG staining. These results suggest that zinc accumulation occurs in the synaptic vesicles of the AC terminals, that TeNT blocks exocytosis of zinc containing presynaptic vesicles, and that an increase in AMG signal can be produced simply as a result of inhibition of the synaptic vesicle cycle of zinc-containing terminals. After ONC and vehicle injection, AMG labeling in the IPL was elevated on day 1 and diminished to near normal levels two days later by day 3. When TeNT was injected immediately after ONC, zinc was also elevated in the IPL on day 1 but remained elevated at day 3, suggesting that dissipation of the ONC-induced zinc accumulation in the IPL occurred between day 1 and day 3 by exocytosis.

Blockage of ONC-induced zinc release by TeNT improved RGCs survival up to 180 % and axon regeneration up to 380 % (at 0.5 mm from the crush site) in a dose-dependent manner. The presynaptic vesicles of inhibitory neurons containing zinc presumably also contain inhibitory neurotransmitter, and further studies will investigate whether the effects of TeNT on RGC survival and axon regeneration after ONC may also require blocking the release of inhibitory neurotransmitters. Taken together, these results indicate a critical role for exocytosis in the disruption of zinc homeostasis that occurs in the retina after ONC.

Disclosures: E.G. Sergeeva: None. **Y. Li:** None. **L.I. Benowitz:** None. **P.A. Rosenberg:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.10/E35

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Methionine metabolism dysregulation in oligodendrocytes contributes to MS phenotype

Authors: *S. STERNBACH, N. SINGHAL, E. FREEMAN, J. MCDONOUGH;
Kent State Univ., Kent, OH

Abstract: Multiple Sclerosis (MS) is characterized by neurological dysfunction and demyelination of the central nervous system. Further, oligodendrocytes are killed off and myelin production is halted, with progenitor cells (OPCs) unable to differentiate. We have previously shown that activation of the BHMT–betaine pathway contributes to epigenetic changes on histone H3 and alleviates neurological deficits in the cuprizone and EAE models of MS. We have found that the enzyme betaine homocysteine methyltransferase (BHMT) is expressed in oligodendrocytes in both the cytoplasm and nucleus. Chromatin fractionation revealed that BHMT is bound to chromatin in oligodendrocytes. Therefore, our lab hypothesizes that through betaine supplementation, the BHMT–betaine pathway locally contributes to SAM synthesis for methylation of DNA and histones in oligodendrocytes. In addition, Seahorse respirometry was performed to determine the effect of betaine on mitochondrial function in oligodendrocytes following oxidative damage. Our data show that betaine modulates oligodendrocyte energetics, increasing glycolysis in mature oligodendrocytes and respiratory capacity in OPCs. These data suggest that changes in methionine metabolism in MS may be linked to defects in oligodendroglial energetics. Thus, activation of the BHMT-betaine pathway may provide epigenetic control required for oligodendrocyte maintenance.

Disclosures: S. Sternbach: None. **J. McDonough:** None. **N. Singhal:** None. **E. Freeman:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.11/E36

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH R56 AG055497

Title: Lysosome-autophagosome defects mediated proteinopathy in early stages of Alzheimer's disease pathogenesis

Authors: *S. H. MUSTALY[1], A. GILMAN-SACHS[2], K. D. BEAMAN[2], J. MCDAID[1], S. SCHRANK[1], R. A. MARR[1], G. E. STUTZMANN[1];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by abnormal protein aggregates and synaptic deficits. To regulate normal cellular processes, lysosomes require an acidic intralysomal pH maintained by the vacuolar H^+-ATPase (V-ATPase). This acidic environment is essential for autophagy, a catabolic pathway to degrade abnormal proteins. In AD, V-ATPase disruption can lead to abnormal β-amyloid and tau accumulation. A concurrent mechanism with V-ATPase defects is intracellular Ca^{2+} dyshomeostasis, which alters ion exchange resulting in alkalinization of pH within this organelle. We hypothesize that Ca^{2+} dyshomeostasis disrupts V-ATPase ion exchange and instigates AD pathology. Here, we used immunoassays and live cell imaging in model cells, induced human neurons (iNs) derived from AD patients, and AD mouse models, to explore this hypothesis. Immunohistochemistry in fixed hippocampal slices from 3-month old 3xTg-AD mice revealed diminished expression of V-ATPase subunits (V1B2, V0a1), lysosomes (Lamp1), and increased expression of mature autophagosomes (LC3B), and hyperphosphorylated tau (p-tau; S262) relative to non-transgenic (NTg) controls. These phenotypes in 3xTg-AD mice were restored to NTg levels with a 30-daytreatment using the ryanodine receptor (RyR) modulator Ryanodex; 10mg/kg). These results were replicated in iNs where increased autophagosomes were shown in iNs derived from AD patients and restored to control levels with Ryanodex treatment. The decreased V-ATPase expression and increased autophagosomes in both models reflect impaired lysosomal function, which is mediated through upstream RyR-Ca^{2+} dyshomeostasis. Live cell imaging of iNs and RyR-overexpressing HEK293 cells using a lysosomal-pH indicator (LysosensorDND-160) revealed lysosomal alkalinization with RyR stimulation (caffeine 10mM). Ryanodex pre-treatment attenuated this alkalinization, suggesting that RyR-Ca^{2+} signaling regulates lysosomal pH and disrupts autophagosome-lysosome mediated protein degradation. Protein aggregates, such as β-amyloid and hyperphosphorylated tau, were increased in iNs treated with 500nM

bafilomycin (V-ATPase inhibitor); these effects were resolved with Ryanodex treatment. Therefore, increased RyR-mediated Ca^{2+} release causes lysosome pH to become more alkaline resulting in abnormal protein aggregation. Prior to overt histopathology or cognitive deficits, abnormal Ca^{2+} signaling in AD disrupts lysosomal function leading to altered autophagic-mediated protein clearance that contributes to AD proteinopathy.

Disclosures: S.H. Mustaly: None. **A. Gilman-Sachs:** None. **K.D. Beaman:** None. **J. McDaid:** None. **S. Schrank:** None. **R.A. Marr:** None. **G.E. Stutzmann:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.12/E37

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Involvement of the complement system in the SAH-induced hippocampal abnormalities

Authors: *G. W. BRITZ, M. A. SHARPE, A. S. REGNIER-GOLANOV, D. S. BASKIN, E. V. GOLANOV;
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Abstract: Overwhelming majority, 95%, of SAH survivors experience various long-term memory and cognitive abnormalities. Atrophy of the temporomesial area observed in SAH survivors is suggestive of the hippocampal abnormalities following SAH. The underlying mechanisms of hippocampal damage following SAH are still not completely understood. Recently it was established that reactive astrocytes are capable to exert the negative effects on neurons through activation of the complement system. We hypothesize that SAH-induced cytokine release activates astrocytic complement system, which may exert negative effect on the hippocampal neurons. To test this hypothesis we explored changes in complement expression in human astrocytic culture and expression of complement components in the post-SAH hippocampus. Normal human astrocytes (NHA) were obtained from Lonza and grown to confluency in Astrocyte Cell Basal Medium in 96-well plates. TNFα, C3 and TNFα combined with C3 were applied to the cultured cells, and 24 hours later NHAs were fixed in ice-cold 4% PFA, washed/permeabilized, and immunohistochemical (IHC) analysis of expression of C1qb, CfB and C3 was carried out using quantitative fluorescent microscopy. TNFα and C3 increased C3 ($p=0.005$) and CfB ($p=0.007$) expression, but failed to do so when applied simultaneously suggesting present of feedback limiting activation of the complement system. In the circle of Willis filament perforation model in mice we explored changes in the levels of C1qB, C3 and CfB in different layers of hippocampal foramtion. Four days following the perforation mice were intracardially perfused, brains were extracted, sliced and processed for IHC. In stratum moleculare lacunosum the C3 levels increased by 21% ($p=0.02$, $n=6$) and levels of C4 increased

by 28% ($p<0.04$, $n=4$) as determined by fluorescent immunohistochemistry. Increase in hippocampal C3 levels was also confirmed by western blot analysis, which demonstrated increase in C3 levels in the whole hippocampus by 30±5 % ($p<0.05$). Comparably, RT-qPCR demonstrated increase in 1.83 increase in C3 expression and 4.8 times increase in C4b expression. These observations confirm our previous observations of increased gene expression of *C3* and *C4* revealed by our transcriptome analysis. The IHC demonstrated striking differences ($p<0.001$) in the complement component expression in the hippocampal layers, which receive different inputs suggesting different roles of complement in functional alterations triggered by SAH depending on diverse the hippocampal inputs.

Disclosures: G.W. Britz: None. **M.A. Sharpe:** None. **A.S. Regnier-Golanov:** None. **D.S. Baskin:** None. **E.V. Golanov:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.13/E38

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Effects in anxiety like behaviour after consumption of two lipid diets in 45 days age old Wistar rats

Authors: *A. CARBALLO-VILLALOBOS[1], P. VERGARA ARAGÓN[2], I. GRACIA MORA[1], A. GÓMEZ-MARTÍNEZ[3], V. MELÉNDEZ PÉREZ[4], H. GARCÍA RODRÍGUEZ[3], A. ZAPATA ARENAS[4], R. BUSTAMANTE-GARCÍA[3];
[1]Inorganic Chem., [2]Med., [3]Biol., [4]Vet. Med., UNAM, Mexico city, Mexico

Abstract: Many studies support the hypothesis that the structure and function of the brain may be modulated by specific aspects of diet, including frequency, content and total energy intake throughout life of the organism. It is know that almost all foods are important in brain tissues, but certain mental faculties require more of certain nutrients for restoration, especially the lipids. The aim of this study was to evaluate two lipid diets, from different sources a vegetable lipid (soybean oil), animal origin (lard) and determine if these modify animal's behavior after 21 days of administration, since the variation of any nutrient could can lead not only physiological but also behavioral disorders, such as anxiety, depression, obsessive compulsive disorders or impaired memory. For this purpose Wistar rats were used (45 ± 50 days), divided into 5 groups (n=6 per group), and were placed in a rack individually, with the following diets: 1= Reference diet, 2= vegetal shortening 3= soy oil, after 21 days animals were evaluated with the task of marble burying behavioral that evaluates anxiety like behavior. We observed that the diet with soy oil the rats show an anxiogenic effect compared with control diet. When we evaluate the vegetable shotering we did not observe any significant change. This data suggest that some kind

of lipids can alter the anxiety like behavior and at a long term affect the process of satiety that could affect the memory and learning process causing a degenerative disease. However in the brain tissues do not exist structural damage wanted in histopathologic studies.

Disclosures: **A. Carballo-Villalobos:** None. **P. Vergara Aragón:** None. **I. Gracia Mora:** None. **A. Gómez-Martínez:** None. **V. Meléndez Pérez:** None. **H. García Rodríguez:** None. **A. Zapata Arenas:** None. **R. Bustamante-García:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.14/E39

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NEI RO1EY027881 (PAR, LIB)
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NINDS RO1NS066019
NIH R21MH104318
2T32EY007145-19
IDDRC HD018655
Adelson Medical Research Foundation (LIB)

Title: Reversal of glutamate transport contributes to retinal zinc elevation and ganglion cell death after optic nerve injury

Authors: *N. J. HANOVICE[1], Y. LI[3], N. C. DANBOLT[4], L. I. BENOWITZ[1], P. A. ROSENBERG[2];
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Abstract: Retinal ganglion cells (RGCs) cannot regenerate their axons after optic nerve crush (ONC) and subsequently die, leading to blindness. The mechanisms linking axonal injury to RGC death remain unclear. Recently, we discovered that retinal interneurons play a critical, previously uncharacterized, role in linking ONC to RGC death in mice: namely, that ONC leads to a rapid increase of mobile zinc (Zn^{2+}) in amacrine cell (AC) terminals, and that chelating Zn^{2+} improves RGC survival and axon regeneration. The signals linking ONC to Zn^{2+} accumulation remain unknown. We previously reported that Zn^{2+} is liberated from intracellular reserves by nitric oxide (NO) that is generated by NO synthase-1 (NOS1) [Li et al. *SfN 2017. #742.09*]. NOS 1 is commonly activated by Ca^{2+} entry through activated NMDA receptors. To determine whether NMDA receptor activation is required for Zn^{2+} accumulation, we injected the NMDA

receptor inhibitor MK801 prior to ONC and found that this prevented Zn^{2+} elevation. Because NMDA receptors are activated by glutamate, we hypothesized that extracellular glutamate may be increased after ONC due to reversal of transport by glutamate transporters. We found that both 1mM DL-TBOA, a general glutamate transport blocker, and 1mM DHK, a relatively specific GLT-1 blocker, prevented Zn^{2+} elevation and enhanced RGC survival. In the retina, GLT-1 is expressed as two major isoforms: GLT-1a and GLT-1b. Immunohistochemistry revealed that GLT-1a is expressed by Müller glia and astrocytes, and that GLT-1b is expressed by cone bipolar cells; neither is expressed in RGCs. We next sought to determine the cellular localization of the GLT-1 responsible for elevating Zn^{2+} after ONC using a conditional GLT-1 knockout. To delete GLT-1 in astrocytes, we used GLAST-CreERT and injected tamoxifen between P7-P14. We found that GLT-1a expression was decreased and GLT-1b expression levels were preserved in GLAST-Cre^{+} mice. In these mice, ONC led to higher Zn^{2+} elevation compared to GLAST-Cre^{-} controls. Using vGlut1-Cre to delete GLT-1 specifically in bipolar cells led to a specific loss of GLT-1b in vGlut1-Cre^{+} mice and, importantly, eliminated Zn^{2+} elevation after ONC. These data suggest that after ONC, GLT-1b expressed by cone bipolar cells contributes to an elevation of extracellular glutamate that activates NMDA receptors, culminating in Zn^{2+} elevation and RGC degeneration. Interestingly, GLT-1a expressed by Muller glia appears to function normally, working to maintain glutamate homeostasis. These results shed further light on the signaling events linking ONC to RGC degeneration, and may facilitate development of treatments for blinding diseases such as glaucoma.

Disclosures: **N.J. Hanovice:** None. **Y. Li:** None. **N.C. Danbolt:** None. **L.I. Benowitz:** None. **P.A. Rosenberg:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.15/E40

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NRF- 2017M3A9G8084464
KGM4621922
KGM5281921
KGM1912

Title: Chronic infiltration of T lymphocytes into the brain in a non-human primate model of Parkinson’s disease

Authors: *J. SEO;
Korea Res. Inst. of Biosci. and Biotech, Cheongju, Korea, Republic of

Abstract: Interactions between the nervous systems and immunity offer clues to Parkinson's disease (PD) pathogenesis and therapeutic strategies for neurodegenerative diseases. Previous rodent and primate studies have revealed different regulatory mechanisms of microglial activation and T lymphocyte recruitment in PD. However, the mechanism underlying chronic T lymphocyte infiltration into the brain after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injection in a non-human primate (NHP) model of PD remains unknown. In this study, we aimed to investigate changes in serum RANTES and analyze the chronic infiltration of T lymphocytes into the brain and microglia activation in NHPs at 48 weeks post-MPTP administration. We found selective and local chronic T lymphocyte infiltration, CD4+ and CD8+ T lymphocyte dopamine transporter expression, chronic normalization of RANTES in peripheral blood, and altered microglial morphology at 48 weeks after MPTP injection. This study confirms the characteristics of MPTP-induced NHP models regarding chronic infiltration of CD4+ and CD8+ T lymphocytes, which are similar to human PD and distinct from rodent models. Further, we corroborated the results of previous studies regarding the mechanisms of T lymphocyte-induced neurodegeneration. Elucidating the mechanism of T lymphocyte infiltration in our MPTP NHP model will assist with further understanding of the neuropathological mechanisms of PD in humans.

Disclosures: J. Seo: None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.16/E41

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Lsm12-Epac1 pathway suppresses C9orf72 poly(GR)-induced neurodegeneration by establishing ran gradient for nucleocytoplasmic transport

Authors: *J. LEE, J. PARK, J.-H. KIM, C. LIM;
UNIST, ULSAN, Korea, Republic of

Abstract: Nucleocytoplasmic transport (NCT) defects have been implicated in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD) associated with *C9orf72* mutations. Here we identify a neuroprotective pathway of *like-Sm protein 12* (*Lsm12*) and *Exchange protein directly activated by cyclic AMP* (*Epac1*) that suppresses NCT dysfunction by *C9orf72*-derived poly(glycine-arginine) proteins. Loss of *Lsm12* function exacerbated neurodegeneration in *Drosophila* models of the poly(GR)-induced ALS/FTD. Consistently, Lsm12 depletion in human neuroblastoma cells enhanced the poly(GR)-induced impairment of NCT while promoting the formation of nuclear poly(GR) granules. Overexpression of ALS-associated Lsm12 mutant comparably strengthened the poly(GR)

toxicity, indicating dominant-negative effects. Transcriptome analyses further revealed that *Lsm12* up-regulates *Epac1* expression whereas Epac1 overexpression rescued NCT defects in Lsm12-deleted cells. In fact, Epac1 depletion dissociated Ran/Importin β1 from cytoplasmic nucleopore complex, thereby dampening Ran gradient. These findings unveil a conserved role of the *Lsm12-Epac1* pathway in the NCT-relevant pathogenesis of *C9orf72*-dependent ALS/FTD.

Disclosures: **J. Lee:** None. **J. Park:** None. **J. Kim:** None. **C. Lim:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.17/E42

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Lipid profiles of exosomes derived from the developing brain exposed to ethanol

Authors: *M. SAITO[1,2], S. CANALS-BAKER[1], K. MASIELLO[1], M. SAITO[1,2], E. LEVY[1,2]; [1]Nathan S Kline Inst., Orangeburg, NY; [2]New York Univ. Langone Hlth., New York, NY

Abstract: Our previous studies have indicated that ethanol exposure in the postnatal day 7 (P7) mice, which has been used for the third trimester model for fetal alcohol spectrum disorders, triggers changes in the brain lipid metabolism, which may be involved in acute apoptotic neurodegeneration observed in these mice. Specifically, ethanol increases brain levels of ceramide (Cer), N-acyl phosphatidylethanolamine (NAPE), triglycerides (TG), and GM2 ganglioside, which are all implicated in the process of cell death. It is believed that exosomes, endosome-derived small extracellular vesicles (EVs), remove toxic materials from cells and/or carry molecules that exert physiological and pathological functions in recipient cells. In this study, we analyzed the effects of P7 ethanol on major lipid profiles in brain exosome-enriched EVs (EVs hereafter), which were isolated as described [Perez-Gonzalez et al., J Biol Chem 2012, 287:43108] from brains removed 1 day after saline/ethanol (2.5 g/kg, twice) injections into P7 mice. Lipids analyses included anandamide (AEA) and 2-arachidonoylglycerol (2-AG), because these endocannabinoids exert numerus functions as signaling lipids, and AEA has been implicated in P7 ethanol-induced neurodegeneration. Levels of AEA and 2-AG were measured by HPLC after dansyl esterification, and levels of other lipids were analyzed by HPTLC. As indicated previously in exosomes from other cell types, we found that sphingolipids were highly enriched in the EVs compared to brain homogenates in both saline (control) and ethanol treated-mice. For example, glucosylceramide was 46±7 times more enriched in the control EVs compared to 7±0.3 times enrichment of cholesterol. Among gangliosides, higher glycosylated molecules (specifically GD1b and GT1b) were more enriched in the EVs (131±10 and 11±0.6 times enrichment in GD1b and GM3, respectively), which may reflect differences in cellular or subcellular localization of each ganglioside. Also, 2-AG was enriched in EVs (26±5 times

enriched), while AEA was not. The P7 ethanol-induced elevation in GM2, Cer, TG, and NAPE was observed in both brain homogenates and the EVs. However, the increase in NAPE (a precursor of AEA) in EVs was 10 times less than that in homogenates, and ethanol increased AEA levels (3.8±0.9 times higher) in the EVs but not in the homogenates. These results indicate that P7 ethanol produces EVs containing higher amounts of apoptosis-related lipids; Cer, TG and AEA. Such EVs may help remove toxic lipids from neurons or transfer the lipids to other cells and spread neurodegeneration.

Disclosures: M. Saito: None. **S. Canals-Baker:** None. **K. Masiello:** None. **M. Saito:** None. **E. Levy:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.18/E43

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Characterizing synapse dysfunction in *Drosophila* mayday mutants

Authors: *J. M. WILLIS, D. WEAVER, D. T. BABCOCK;
Lehigh Univ., Bethlehem, PA

Abstract: Maintaining synaptic structure and function overtime is vital for overall nervous system function and survival. Recent evidence has implicated synaptic defects in aging and neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), and Parkinson's disease (PD). These diseases are characterized by the loss of function in neurons. Our current understanding of neurodegeneration has come from late stage or postmortem studies. Existing treatments seek to target these diseases by alleviating symptoms. New evidence has shown that synapses begin to deteriorate long before the neurons die, suggesting that there are earlier events that could be targeted. However, it remains unclear how synapses degenerate. To understand neurodegeneration in *Drosophila*, we have developed a high throughput behavioral flight assay that has been used to screen mutant flies for an impaired flight phenotype. The impaired phenotype suggests issues with motor neurons or associated muscles. From our preliminary flight screen, we isolated a mutant in a previously novel uncharacterized gene, CG31475. *Cab45* is the mammalian homolog to CG31475 and is involved with cargo sorting at the Trans-Golgi Network. Therefore, we seek to elucidate the role of CG31475, now referred to as "*mayday*" and its role in synapse dysfunction in *Drosophila.*

Disclosures: J.M. Willis: None. **D. Weaver:** None. **D.T. Babcock:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.19/E44

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Children's Tumor Foundation 2015-04-009A
NIH R01NS082283

Title: The role of neurofibromin in neurocognitive impairment, CNS cell function and differentiation in a porcine model of neurofibromatosis type 1 (NF1)

Authors: *V. J. SWIER[1], K. A. WHITE[1], P. NEGRAO DE ASSIS[1], A. MOUTAL[3], R. KHANNA[3], J. M. WEIMER[2];
[2]Children's Hlth. Res. Ctr., [1]Sanford Res., Sioux Falls, SD; [3]Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Loss of the *NF1* tumor suppressor gene causes the autosomal dominant condition, neurofibromatosis type 1 (NF1). Children and adults with NF1 suffer from pathologies including benign and malignant tumors to cognitive deficits, seizures, growth abnormalities and peripheral neuropathies. Existing NF1 mutant mice mimic individual aspects of NF1, but none comprehensively models the disease. We have characterized a novel Yucatan miniswine model bearing a heterozygotic mutation in NF1 (exon 42 deletion) orthologous to a mutation found in NF1 patients. The $NF1^{+/ex42del}$ miniswine phenocopy the wide range of manifestations seen in NF1 patients, including café au lait spots, neurofibromas, axillary freckling, and neurological defects in learning and memory. We continue to investigate the brain dysfunction and cognitive impairments by longitudinal phenotypic monitoring of tumor development, social behavior and activity as well as characterization of neurocognitive impairment through the use of a T-maze. As one of the common symptoms of neurofibromatosis is chronic idiopathic pain, we compared sensitivity to stimuli in the $NF1^{+/ex42del}$ miniswine. To better understanding how mutations in neurofibromin effect unique cell types of the CNS, we are carefully examining the morphology and cellular changes in astrocytes, oligodendrocytes and specific classes of neurons that have previously suggested to be altered in NF1. Moreover, we describe disruptions in locomotor activity, learning and memory, and sensitivity to stimuli over time in $NF1^{+/ex42del}$ swine. We have a novel NF1 porcine model that recapitulates characteristics seen in NF1 patients that have not been identified in mouse models, allowing careful mapping of the changes in specific neural circuits or brain regions that may contribute to the neurological deficits of NF1 .

Disclosures: V.J. Swier: None. **K.A. White:** None. **P. Negrao de Assis:** None. **A. Moutal:** None. **R. Khanna:** None. **J.M. Weimer:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.20/F1

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: 5T32DK101357-03
R24DK082841

Title: Nailing down peripheral neuropathy; a lipidomics approach

Authors: S. E. ELZINGA[1], A. E. RUMORA[1], L. M. HINDER[2], K. GUO[3], S. EID[1], P. O'BRIEN[1], J. M. HAYES[1], M. A. TABBEY[1], J. HUR[3], *E. L. FELDMAN[1];
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Abstract: Increasing rates of obesity, prediabetes, and diabetes are contributing to similar increases in rates of complications, including neurological complications such as peripheral neuropathy (PN). PN is estimated to effect 30 to 60% of patients with prediabetes and diabetes. Controlling glucose has little effect on the onset and progression of PN in prediabetes or type 2 diabetes (T2D). Recent clinical studies suggest rather than hyperglycemia it is dyslipidemia that underlies the pathogenesis and/or progression of PN. To begin to understand how dyslipidemia underlies PN, we compared lipidomic profiles in plasma and in sciatic nerve across commonly utilized mouse strains (BL6, BTBR, and BKS) of 36 week old male mice fed a high fat diet compared to animals fed a standard diet to better underlying mechanisms of lipid dysregulation in PN and to identify possible biomarkers of PN. When comparing lipid species effected by high fat feeding, we observed little agreement between mouse strain or between sciatic nerve and plasma. Compared to other strains tested and as previously published, BL6 mice had the most robust metabolic and neuropathic phenotype in response to high fat feeding. Within BL6 mice, we observed an upregulation in the nerve of triglycerides and a down regulation of various phospholipids in response to high fat diet. In the plasma, phospholipid species were also down regulated. Collectively, these data suggest that elevated nerve triglycerides and lowered phospholipids constitute the neural signature in PN, and that lowered plasma phospholipids could be a biomarker of the diseased state.

Disclosures: S.E. Elzinga: None. **A.E. Rumora:** None. **L.M. Hinder:** None. **K. Guo:** None. **S. Eid:** None. **P. O'Brien:** None. **J.M. Hayes:** None. **M.A. Tabbey:** None. **J. Hur:** None. **E.L. Feldman:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.21/F2

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Preventing aggregation of multiple misfolded proteins and promoting their clearance in neurodegeneration

Authors: D. IBGHI, *P. BERTRAND, Y.-J. WU, T. CHEVET, N. VAUCHER, A. CEDAZO-MINGUEZ;
Rare & Neurologic Dis. Res. Therapeut. Area, SANOFI R&D, Chilly-Mazarin, France

Abstract: Abnormal protein accumulation in the brain is a common pathological hallmark in patients suffering from neurodegenerative diseases (NDDs). Amyloidopathies, tauopathies and synucleinopathies are characterized by the aggregation of misfolded proteins (e.g., beta-amyloid, tau, alpha-synuclein (aSyn), TDP43, prion proteins, insulin⋯) into fibrils that ultimately compose these protein deposits.
Impaired clearance also contributes to the accumulation of these proteins and, in association with insufficient neuroprotective mechanisms, participates in the progression of pathological conditions leading to neurodegeneration. In exploratory studies, we have investigated the effects of several novel peptidic compounds. These polypeptides were able to inhibit the aggregation of multiple proteins (i.e., Aβ42, tau, aSyn, insulin) *in vitro* using cell-free Thioflavin T assay or intracellularly as measured by HTRF. Remarkably, some of these compounds had neuroprotective effects, and were able to enhance intracellular degradation of amyloidogenic proteins, in primary cell cultures from embryonic C57BL/6 mouse (cortex and hippocampus neurons and microglia) or in cell lines expressing human mutated Tau. These results suggest a dual protective mechanism of these compounds by:
-preventing de novo formation of protein aggregates that accumulate in patients' brains
-increasing intracellular clearance of preformed cytotoxic components
Promoting such combined multi-target activity is thought to be a potential therapeutic approach to slow down the pathogenesis and the progression of NDDs.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.22/F3

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: VA merit

Title: Pathological assessment in heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) in multiple sclerosis

Authors: *S. LEE[1,2], Y. SHIN[1,2], J. W. TSAO[1,2], M. C. LEVIN[3];
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Abstract: The heterologous nucleolus ribonucleoprotein A1 (hnRNP) A1, which is involved in various RNA metabolism, changes in the degree of expression in a neurodegenerative disease including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) or in the mutation of the hnRNP A1 gene which is known to be involved in the development of ALS and multisystemic proteinopathy. It is also known that excessive expression of low grade hnRNP A1 induces cytotoxicity by activation of the mitochondrial apoptotic pathway. Thus, rigorous quantitative and qualitative control of hnRNP A1 under physiological conditions is required. For pathological evaluation of hnRNP A1 in multiple sclerosis (MS), we examined the cell distribution of hnRNP A1 from motor neurons in the brain tissue of MS patients, including the control group. The pathologic features of hnRNP A1 in MS patients were depleted from the nucleus and significantly lost in the cytoplasm, similar to amyotrophic lateral sclerosis (ALS). In MS, TDP-43 immunoreactivity in motor neurons was reduced in the nucleus of neurons, but skein-like inclusion was not detected in cytoplasm. Therefore, the pathological features of MS are thought to have a dual mechanism different from that of ALS. We further investigated the cell distribution of hnRNP A1 in cell systems (*in vitro*) and the TH17 EAE model (*in vivo*), an animal model of MS. We treated transcription inhibitor (actinomycin D or staurosphorin), oxidative stress inducer (arsenite), shRNA, cytokines, and mutated hnRNP A1 in SK-N-SH neuroblastoma cells. Human primary neurons were also treated with Th1 or Th17-related cytokines for pathological evaluation of hnRNP A1. Brains and spinal cords were collected from four Th17 EAE model groups (-3 days, 5 days, 13 days, 21 days) before and after injection of Th17 cells and cell distribution of hnRNP A1 was examined. Analysis to date show that hnRNP A1 is depleted from the nucleus and aggregates in the cytoplasm and undergoes proteolytic activity under transcription inhibition conditions, whereas proteolysis does not occur under oxidative stress conditions. These proteolytic activities were also confirmed to occur in the central nervous system (CNS) tissues of the EAE model. Thus, the loss of motor neurons in MS

is thought to be an important pathway for transcriptional inhibition, and a significant loss of hnRNPA1 in motor neurons may represent a severe impairment of mRNA stability, suggesting a key role in MS neuronal cell death.

Disclosures: **S. Lee:** None. **Y. Shin:** None. **J.W. Tsao:** None. **M.C. Levin:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.23/F4

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH R01NS082283

Title: BioID identifies CLN3 protein interactions

Authors: ***C. SWANSON**[1], T. B. JOHNSON[1], J. J. BRUDVIG[1], D. G. MAY[1], K. J. ROUX[1], J. M. WEIMER[2];
[2]Children's Hlth. Res. Ctr., [1]Sanford Res., Sioux Falls, SD

Abstract: CLN3-Batten Disease is a rare autosomal pediatric neurodegenerative disorder caused by mutations in *CLN3*, which encodes a putative lysosomal transmembrane protein. CLN3-Batten disease is characterized by visual deficits leading to complete blindness, seizures, motor abnormalities, ataxia, dementia, and premature death by the third decade of life. Despite thorough characterization of disease pathology and progression, the exact function of CLN3 is still vastly unknown. A more basic understanding of how CLN3 disruption affects the different cell types and how different protein-protein interactions are compromised is needed in order to identify the molecular mechanisms disrupted as a result of *CLN3* mutations. In order to address this critical need, we developed a lysosomal BioID paradigm that labels proteins that interact with CLN3 at the lysosomal interface by attaching a promiscuous mutant biotin ligase (BirA) to CLN3 protein at the cytosolic N-terminus. We transduced Neuro2A cells with a retrovirus containing the *CLN3-BirA* fusion and through proximity dependent biotinylation these experiments identified proteins that have proximal interactions with the CLN3 protein. Additionally, we designed and synthesized AAV9-*CLN3-BirA* virus for *in vivo* transduction of neurons in mice to elucidate *in vivo* interactions of CLN3, specifically in axons and dendrites. These interacting proteins were captured, purified, and identified using mass spectrometry. By defining the interactome of the CLN3 protein, we can further our understanding of the function of CLN3, thus allowing us to gain insights into novel therapeutic targets for neurodegenerative disease.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.24/F5

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Packard Center for ALS Research
ALS 16-IIP-278
MDA 348086
RO1 NS085207
Barrow Neurological Institute

Title: ADAR2 mislocalization and widespread RNA editing aberrations in C9orf72-mediated ALS/FTD

Authors: *S. P. MOORE[1], E. ALSOP[2], I. LORENZINI[1], A. STARR[1], B. E. RABICHOW[1], J. L. LEVY[1], C. BURCIU[1], R. REIMAN[2], J. CHEW[3], V. BELZIL[3], D. W. DICKSON[3], J. ROBERTSON[4], K. A. STAATS[5], J. K. ICHIDA[5], L. PETRUCELLI[3], K. VAN KEUREN-JENSEN[2], R. SATTLER[1];
[1]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [2]The Translation Genomics Res. Inst., Phoenix, AZ; [3]Neurobio. of Dis. Grad. Program, Mayo Clin., Jacksonville, FL; [4]Tanz Ctr. for Res. Neurodegenerative Dis., Univ. of Toronto, Toronto, ON, Canada; [5]Stem Cell Biol. and Regenerative Medicine, Keck, Los Angeles, CA

Abstract: The hexanucleotide repeat expansion GGGGCC $(G_4C_2)_n$ in the *C9orf72* gene is the most common genetic abnormality associated with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Recent findings suggest that dysfunction of nuclear-cytoplasmic trafficking could affect the transport of RNA binding proteins in C9orf72 ALS/FTD. We have discovered evidence that the RNA editing enzyme adenosine deaminase acting on RNA 2 (ADAR2) is mislocalized in C9orf72 repeat expansion mediated ALS/FTD. ADAR2 is responsible for adenosine (A) to inosine (I) editing of double-stranded RNA, and its function has been shown to be essential for survival. Here we show the mislocalization of ADAR2 in human induced pluripotent stem cell-derived motor neurons (hiPSC-MNs) from C9orf72 patients, in mice expressing $(G_4C_2)_{149}$, and in C9orf72 ALS/FTD patient postmortem tissue. As a consequence of this mislocalization we observe alterations in RNA editing in our model systems and across multiple brain regions. Analysis of editing at 408,580 known RNA editing sites indicates that there are vast RNA A to I editing aberrations in C9orf72-mediated ALS/FTD. These RNA editing aberrations are found in many cellular pathways, such as the ALS pathway

and the crucial EIF2 signaling pathway. Finally, we can induce similar RNA editing abnormalities by overexpression of a ΔNLS-ADAR2 that is restricted to the cytoplasm. Our findings suggest that the mislocalization of ADAR2 in C9orf72 mediated ALS/FTD and its aberrant presence in the cytoplasm is responsible for the alteration of RNA processing events that may impact vast cellular functions, including the integrated stress response (ISR) and protein translation.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.25/F6

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: National Institute of Neurological Disorders and Stroke (R51NS095317)

Title: Bag6 prevents the aggregation of neurodegeneration-associated protein fragments

Authors: *Y. T. K. KASU, J. JOHNSON, C. SAJAN, C. S. BROWER;
Biol., Texas Woman's Univ., Denton, TX

Abstract: The accumulation and aggregation of misfolded proteins is characteristic of many neurodegenerative disorders. This results, at least in part, from defects in protein quality control systems such as chaperone function or degradation by the ubiquitin proteasome system (UPS). During pathological conditions, many neuronal proteins are susceptible to endoproteolytic cleavage resulting in protein fragments (PFs) that are also aggregation-prone due to exposure of their hydrophobic regions. Previously, we found that the N-degron pathway of the UPS degrades a number of specific disease-associated PFs, including those of the TAR DNA binding protein 43 (TDP43) that are associated with amyotrophic lateral sclerosis and frontotemporal lobar degeneration [1]. Specifically, we found that PFs of TDP43 accumulate and aggregate in cells lacking *ATE1* gene function. In a follow-up study, we found that differences in the N-termini of otherwise identical PFs influence their degradation, aggregation propensity and aggregate morphology [2]. Here, we report that BAG6, a molecular chaperone component of a cytosolic protein quality control complex, can recognize hydrophobic regions in PFs and prevent their aggregation. This suggests that BAG6 may prevent neurodegeneration associated with PF aggregation.
1. Brower, C.S., et al., *Neurodegeneration-associated protein fragments as short-lived substrates*

of the N-end rule pathway. Mol Cell, 2013. **50**(2): p. 161-71.
2. Kasu, Y.A.T., et al., *The N-termini of TAR DNA-binding protein-43 (TDP43) C-terminal fragments influence degradation, aggregation propensity and morphology.* Mol Cell Biol, 2018. **38**(19): p. e00243-18.
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Disclosures: Y.T.K. Kasu: None. **J. Johnson:** None. **C. Sajan:** None. **C.S. Brower:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.26/F7

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: The role of inflammation underlying vision impairment after traumatic brain injury

Authors: *L. P. EVANS[1], A. WOLL[3], S. WU[1], E. A. NEWELL[4], P. J. FERGUSON[2], V. MAHAJAN[5], M. HARPER[3], A. G. BASSUK[1];
[2]Dept. of Pediatrics, [1]Univ. of Iowa, Iowa City, IA; [3]The Iowa City Dept. of Veterans Affairs Ctr. for the Prevention and Treatment of Visual Loss, Iowa City, IA; [4]Pediatrics, Univ. of Iowa Carver Col. of Med., Iowa City, IA; [5]Dept. of Ophthalmology, Stanford Univ., Palo Alto, CA

Abstract: PURPOSE: Blast-mediated traumatic brain injury (bTBI) affects both military members and civilians with no current effective pharmacologic therapies. Following TBI, secondary signaling cascades occur in the brain including robust neuroinflammation exacerbating the initial neuronal insult. Modulation of the IL-1 pathway, a major inflammatory effector, is a potential therapeutic strategy for treating TBI. The retina is a central nervous system (CNS) tissue that is vulnerable to blast exposure. Individuals with bTBI often report visual dysfunction, yet limited attention has been given to ocular inflammation. Utilizing a murine bTBI model we aim to assess retinal manifestations of CNS injury and characterize acute molecular changes leading to long-term damage that can be targeted therapeutically. METHODS: A compressed air-driven shock tube system exposed wild-type C57BL/6 mice to a blast wave pressure of 20 [Symbol] 0.2 psi (137.8 [Symbol] 1.3 kPa) 3 times, each 1 hr apart. Acute cytokine expression in retinal tissue was measured via RT-PCR and histologic activation of retinal microglia and astrocytes was assessed 4 hr and 1 wk post-blast, respectively. Mice treated with anakinra, an IL-1R1 antagonist, underwent pattern electroretinograms and optical coherence tomography to assess retinal ganglion cell (RGC) function and structure, respectively. Optic nerves (ON) were assessed for neurodegeneration. REULTS: Increased retinal expression of inflammatory markers lL-1[Symbol], IL-1[Symbol], IL-6, and TNF[Symbol] were seen in bTBI mice when compared to shams. bTBI mice showed an enhanced response of microglia and

astrocytes in the retina due to the injury stimulus. Mice treated with anakinra exhibited a partial rescue of RGC function, decreased RGC death, and reduced damage in their ON.
CONCLUSIONS: bTBI causes an inflammatory response in the retina with increased expression of pro-inflammatory cytokines and activation of resident microglia and astrocytes, which could contribute to RGC loss in this model. Blockade of the acute inflammatory response after injury via anakinra resulted in partial preservation of the RGC function and structure that is normally lost due to blast injury. These preliminary studies could result in repurposing currently approved pharmaceuticals for bTBI patients.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.27/F8

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: R01GM088801
R01HD 086977

Title: Microbiome dysbiosis and postoperative delirium pathogenesis

Authors: *Y. ZHANG, L. LIU, N. LIUFU, Z. XIE;
Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA

Abstract: OBJECTIVE: Postoperative delirium (POD) is a common but often undiagnosed complication following a major surgery, which is a quintessential geriatric complication. POD is defined as an acutely altered and fluctuating mental status with features of inattention and an altered level of consciousness. The recognition and treatment of POD is critically important because it is associated with functional decline, longer hospitalization, greater cost of care, as well as higher morbidity and mortality. However, the pathogenesis of POD is still largely unknown. Only subsets of patients (e.g., senior patients) develop POD, the reason behind this clinical observation is largely unknown. Aging is known to be associated with marked microbiome changes and microbiome dysbiosis links with disorder in immune, endocrine, and nervous system. We therefore set out to assess anesthesia/surgery caused age-dependent changes in delirium-like behavior, brain mitochondrial function in mice, and the microbiome-associated underlying machnism.
METHODS: We performed abdominal surgery under 1.4% isoflurane in mice for 2 hours in both 9 month-old and 18 month-old WT mice. We then measured: (1) gut microbiome (16s rRNA

gene sequencing) before and after the anesthesia/surgery; and (2) mitochondrial function (Seahorse XFp Extracellular Flux Analyzer); and (3) POD-like behavior, a battery of behavior tests. Composite Z scores were calculated. We also use lactobacillus and probiotic drops to treat the mice before anethesia/surgery and detect the above changes.
RESULTS: Anesthesia/surgery induces POD-like behavior in mice. The aged mice (18 months-old) have significantly larger composite Z-scores compared to adult (9 months-old) mice at 6 and 9 hours, but not 24 hours after the anesthesia/surgery. Anesthesia/surgery decreases oxygen consumption rate (OCR) and induce a greater reduction in OCR in aged mice and causes greater brain mitochondrial dysfunction in aged mice. Finally, anesthesia/surgery decreases the levels of gut lactobacillus in the aged mice, moreover, lactobacillus and probiotic drops both mitigates mitochondrial dysfunction and the POD-like behavior in the aged mice induced by anesthesia/surgery.
CONCLUSIONS: We have showed the age-dependent changes in gut microbiome, mitochondrial function, and POD-like behavior and revealed the mitigation effects of lactobacillus in the POD-like behavior in the mice. Clinically, these efforts may challenge the current practice and provide better postoperative outcomes for POD patients.

Disclosures: **Y. Zhang:** None. **L. Liu:** None. **N. Liufu:** None. **Z. Xie:** None.

Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.28/F9

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH/NIAID R01AI13241401A1

Title: Identifying mechanisms underlying neural dysfunction in iPSC derived cortical organoids infected with human cytomegalovirus

Authors: B. S. O'BRIEN[1], S. L. SISON[1], M. SCHUMACHER[2], S. S. TERHUNE[3], *A. D. EBERT[1];
[1]Cell Biology, Neurobio. and Anat., [2]Dept. of Microbiology and Immunol., [3]Dept. of Microbiology and Immunology; Dept. of Biomed. Engin., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Human cytomegalovirus (HCMV) is a beta herpesvirus that may result in congenital birth defects upon infection during pregnancy including microcephaly, vision loss, and hearing loss. Currently, no approved treatment options exist for managing *in utero* infections. Our previous work has shown that HCMV infection in undifferentiated human induced pluripotent stem cell (iPSC) derived neural progenitor cells (NPCs) and iPSC derived cortical organoids

have significantly reduced calcium response to ATP and KCl stimulation 5 days post infection. Moreover, HCMV infection substantially alters organoid structure such that rosette formation is impaired, distribution of Pax6 and Sox2 positive cells are disrupted, and expression of the early cortical layer marker Ctip2 is reduced. These data suggest that HCMV causes substantial disruptions in typical neural development and function. However, the mechanisms underlying these results are not well understood. Previous studies in fibroblasts show that HCMV decreases connexin-43 expression. Connexin-43 is a key gap junction protein critical to cell-cell communication, and we show a similar reduction in connexin-43 fluorescence intensity in iPSC-derived cortical organoids compared to mock infected organoids. Decreases in connexin-43 expression are linked to decreases in purinergic receptor and ion channel expression thereby altering cellular signaling pathways. Additionally, we have found that HCMV infection in NPCs causes an increase in expression of the acetyltransferase Tip60, which has roles in the cell cycle and signal transduction. Reduced Tip60 may contribute to the lack of more terminally differentiated neural subtypes in HCMV infected organoids. Finally, to get a more global assessment of the impact of HCMV infection, we are using FACS to isolate HCMV infected cells in the early and late stages of infection followed by RNA sequencing to identify potential pathways contributing to altered neural differentiation, migration, signaling, and function. Together our studies demonstrate that HCMV infection significantly impairs neural function and structure of a 3D organoid potentially due to expression changes in genes involved in signal transduction and cell-cell communication.

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Poster

047. Cellular Stress and Death Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 047.29/F10

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Strategic Priority Research Program B of the Chinese Academy of Sciences (XDBS1020100)
National Science Foundation of China (NSFC No 31110103907 and 31490590)
NSFC grant No 31500824

Title: Ttm50 facilitates calpain proteolytic activity by localizing calpain to calcium stores and increasing calpain sensitivity to calcium

Authors: *E. METWALLY, G. ZHAO, Q. WANG, Y. Q. ZHANG;
State Key Lab. for Mol. and Developmental Biology, CAS Ctr. for Excellence in Brain Sci. and

Intelligence Technology, Inst. of Genet. and Developmental Biology, Chinese Acad. of Sciences., Beijing, China

Abstract: Calpains are a family of cytosolic cysteine proteinases that are implicated in a variety of biological processes, as well as injury and neurodegeneration associated with ischemia and Alzheimer's disease. Calpain proteolytic activity depends on calcium, but the mechanisms whereby this activity is regulated remains elusive. In our previous study, we showed that calpains specifically cleave glutamate receptor subunit GluRIIA but not GluRIIB at the *Drosophila* neuromuscular junction (NMJ) synapses (Metwally et al., *J. Neurosci*, 2019). Here, we show that calcium-dependent activation of calpain induced GluRIIA cleavage was inhibited upon knockdown of Ttm50, which is previously documented to be involved in the transport of proteins across the mitochondrial inner membrane. To our surprise, we found that calpain and Ttm50 are enriched at NMJ synapses as well as at the calcium stores of Golgi and endoplasmic reticulum. Further biochemical and biophysical analysis revealed that Ttm50 is co-localized and interacts with calpain via their C-termini. This interaction increased calcium sensitivity of calpain and was required for calpain localization at the calcium stores to facilitate calpain activation. Our findings reveal a novel regulatory mechanism of calpain activation via Ttm50 in physiology and may shed new light on calpain-associated pathologies.

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Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.01/F11

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2016M3C7A1914123)
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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 number : HI19C0060).

Title: Increased transplantation efficacy of mesenchymal stem cell by focused ultrasound and improvement of the spatial memory in the 192 IgG-saporin rat model

Authors: *J. LEE[1,2], Y. SEO[1,2], J. SHIN[1,2], C. KONG[1], Y. NA[3], W. CHANG[1], J. CHANG[1,2]; [1]Dept. of Neurosurg., [2]Brain Korea 21 PLUS Project for Med. Sci., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [3]Dept. of Neurosurg., Catholic Kwandong Univ. Col. of Med., Incheon Metropolitan City, Korea, Republic of

Abstract: Introduction: Stem cell therapy has been found to have therapeutic effects in neurodegenerative disease, but traditional transplant methods, such as parenchymal or intravenous injection, possess limitations like secondary injuries, infection, and low survival rate of stem cells in the brain. Meanwhile, recently the focused ultrasound(FUS) was found to have promising results regarding transplantation of stem cells into the rat brain. However, the mechanism of stem cell transplantation with FUS and possibility of cognitive recovery remain elusive. Therefore, this study investigates a possibility for non-invasive focused ultrasound use in stem cell transplantation into the brain of dementia rat model. **Materials & methods:** We divided rats into five groups: Normal, Lesion, Cell only, FUS + Cell, and FUS only. We used 192 IgG-saporin for degeneration of basal forebrain cholinergic neuron and it was injected into all rats except for the normal group. After a week, 5p mesenchymal stem cells (MSC: $3*10^6$/200ul) were injected in the tail vein of all rats of the cell only and FUS + Cell group, and the FUS + Cell group received the FUS three hours before cell transplantation. FUS was applied with parameters of 0.25Mpa, 300s (Targeted hippocampal region: AP -3.5, ML ±2). And last, FUS only group was received only FUS without any treatment. Five weeks after transplantation, rats performed the Morris water maze test. **Results:** MSC were detected in both cell only and FUS + Cell group of the hippocampus region. After comparing FUS+MSC & cell only rats, it was confirmed that FUS increases MSC homing in the sonicated rat's brain. In addition, the most effective memory restoration occurred in the FUS + Cell group. Moreover, the FUS + Cell group exhibited better recall of the platform position than the other groups. And FUS only group did not recover. **Conclusion:** Noninvasive FUS can increase the efficacy of stem cell delivery. And memory impairment due to cholinergic denervation can be effectively improved by cell transplantation with FUS. The results of this study suggest possibility of stem cell homing and therapeutic effects of the FUS in dementia rat model. However, further study regarding the function of stem cells transplanted in the brain and a more detailed mechanism of stem cell homing by FUS is needed.

Disclosures: J. Lee: None. **Y. Seo:** None. **J. Shin:** None. **C. Kong:** None. **Y. Na:** None. **W. Chang:** None. **J. Chang:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.02/F12

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2016M3C7A1914123)
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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 number : HI19C0060).

Title: Molecular changes associated with noninvasive transplantation and homing of mesenchymal stem cells by focused ultrasound in the rat brain

Authors: *Y. SEO[1,2], J. LEE[1,2], J. SHIN[1,2], C. KONG[1], Y. NA[3], W. CHANG[1], J. CHANG[1,2]; [1]Dept. of Neurosurg., [2]Brain Korea 21 PLUS Project for Med. Sci., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [3]Dept. of Neurosurg., Catholic Kwandong Univ. Col. of Med., Incheon Metropolitan City, Korea, Republic of

Abstract: Introduction In many animal studies, stem cell therapy has been proven to be an effective treatment for neurodegenerative diseases. Mesenchymal stem cells (MSCs) have great potential as a source of cells for cell-based therapy because of their ease of isolation, immune capability, expansion, proliferation and differentiation potency. The intravenous route is used to deliver stem cells to the brain, but there is a disadvantage that large amounts of stem cells are trapped in the lungs. Meanwhile, some studies have shown that when focused ultrasound(FUS) is used to open the blood-brain barrier(BBB), a variety of cytokines related to stem cell migration are released. Furthermore, there have been reports that increase of matrix metalloproteinase (MMP) 2 and 9 affect to stem cell migration. Therefore, in this study, the basis for the MSC transmigration was investigated related to Matrix metalloproteinase. **Materials and methods** The study used male Sparague-Dawley rats (200-220g). They were divided into six groups, which are normal, FUS-only(sacrificed 1hour, 2hour, 3hour after FUS), FUS+MSC, MSC-only group. FUS-only group and FUS+MSC group were sonicated with parameters of 0.25Mpa, 300s targeting the hippocampal region (AP -3.5, ML ±2). In FUS+MSC group, after three hours of sonication, 5 passages bone marrow-derived MSC ($3*10^6$/200ul) were injected into the tail vein. FUS-only group was sacrificed at 1hour, 2hour, 3hour after FUS, respectively. MSC-only group was injected with MSC into the tail vein. And rats of MSC-only, FUS+MCS group were sacrificed twenty-four hours after MSC injection. **Results** As a result of comparison between FUS+MSC and MSC-only group's sonicated brain region, it was confirmed that MSC homing was increased more in FUS+MSC group than MSC-only group. In addition, several factors related to stem cell homing process such as MMP2 and 9, were altered in the sonicated brain tissue. It is known that MMP 2 and 9 degrade collagen and gelatin which is the main factors of the base membrane of BBB. There were also changes in MMP2 and 9 when comparing the sacrificed groups at 1hour, 2hour, 3hours after FUS with the normal group. **Conclusion** Focused

ultrasound is a way to target specific brain area non-invasively and delivery stem cells safely and effectively. In addition, through the overexpression of MMP-2 and MMP-9, it can be seen that the more stem cells actually passed the blood-brain barrier. The results of this study demonstrate the transmigration of MSC through the use of low intensity focused ultrasound. However, further studies are needed on mechanisms that regulate the expression and effective role of MMPs in the differentiation of MSCs.

Disclosures: **Y. Seo:** None. **J. Lee:** None. **J. Shin:** None. **C. Kong:** None. **Y. Na:** None. **W. Chang:** None. **J. Chang:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.03/F13

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: 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 number : HI19C0060).
NRF-2016M3C7A1914123
NRF-2016R1D1A3B03932649

Title: Changes in the level of P2X7 expression after focused ultrasound induced blood-brain barrier opening in the rat brain

Authors: *J. SIM[1,2], J. SHIN[1,2], C. KONG[2], J. LEE[1,2], Y. NA[3], W. CHANG[2], J. CHANG[1,2]; [1]Brain Korea 21 Plus Project Med. Sci., [2]Dept. of Neurosurg., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [3]Dept. of Neurosurg., Catholic Kwandong Univ. Col. of Med., Incheon Metropolitan City, Korea, Republic of

Abstract: Introduction: Focused ultrasound (FUS) combined with microbubble has been known to produce transient, reversible and non-invasive blood-brain barrier (BBB) opening. In addition, FUS also induces changes of the several cellular signaling pathways. Whether or not FUS induces the sterile inflammation is still under investigation. To investigate changes in inflammatory responses after FUS, several pro-inflammatory and anti-inflammatory factors have been reported. Activation of P2X7, the ligand gated cation channel, changes the intracellular ion homeostasis and forms non-selective pores up to 900Da, allowing migration of immune cells. In this study, we investigate the changes in the expression level of P2X7 at different time point after FUS *in vivo*. **Materials and methods**: Male Sprague-Dawley rats (280~300g) were used in this study. Sonication was performed using a single-element transducer with a frequency of 0.5MHz. The acoustic parameters used for each sonication are pressure amplitude 0.27MPa, burst duration

10ms, pulse repetition frequency 1Hz, and duration of 120s. Before the sonication, microbubble was injected into the tail vein of the rats. The target region is the hippocampal area at the position of 3.5mm posterior and 2mm lateral from bregma. Rats were sacrificed at different time points after sonication for analysis. The control group has not experienced any treatments. **Result:** Compared with the control group, the P2X7 expression level was significantly increase after FUS sonication in hippocampus. This effect was temporary, which means it will be decreased lower than normal level after BBB closed. **Conclusion:** Our study suggested that the expression level of P2X7 is changed by FUS. It was confirmed that the expression level was not simply increased but lower than the normal level after a specific time points after FUS. That means, it will be changed in the immune response triggered by P2X7. For the further study, we confirmed that how P2X7 mediated immune responses changed by FUS.

Disclosures: **J. Sim:** None. **J. Shin:** None. **C. Kong:** None. **J. Lee:** None. **Y. Na:** None. **W. Chang:** None. **J. Chang:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

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Program #/Poster #: 048.04/F14

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: UD140069ID
NRF-2018R1D1A1B07046189
NRF-2017R1D1A1B03034480

Title: Rat movement control using fully implantable neural stimulator

Authors: *C. KOH[1], J. SHIN[1,2], C. KONG[1], S. YUN[3], J. SEO[3], G. CHOI[3], S.-H. AHN[3], S. SHIM[3], H. JUNG[1], S. KIM[3], J. CHANG[1,2];
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Abstract: Introduction: A fully implantable and wireless device is necessary to freely manipulate animal movement. Especially for small sized animals such as rats, the device had to be miniaturized but there was limitation in reducing the size due to its size of the battery. However, the device can be disassembled so it can be easily implanted in the different parts of animal body. Matrials and Methods: We developed a fully implantable neural stimulator in order to manipulate free movement. The stimulator consists of three major modules, package, electrodes, and connections. The package has wireless transceiver, inductive link, and stimulation pulse generator. The electrodes were microfabricated based on LCP and connected to the package by cables and connectors. The device has ZigBee telemetry and inductive charging

of a battery which is the main power source of the stimulator. We conducted *in vitro* tests such as electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) assessments, and wireless operation test in saline solution to ensure the functionality of the stimulator. *In vivo* test was conducted to verify its efficacy and stability. The deep brain electrode was implanted in the medial forebrain bundle (MFB) to deliver virtual reward stimulation. Y-shaped surface electrode was placed in the epidural space of bilateral barrel cortex to present directional cues. The main part of the device was inserted right under the abdominal skin and specially designed polyimid cable connected the two electrodes and the main device. Results: After recovery from the surgery, rats were trained to follow the left or right directional cues. MFB stimulation was delivered to reward rats after proper movement. Rats could rotate either right or left direction following sequent electrical stimulation. After then, we successfully manipulated rat movement in a customized 3D complex maze. Conclusion: This study proved our device is fully functioning and implantable neural stimulator for manipulation of animal behavior.

Disclosures: C. Koh: None. **J. Shin:** None. **C. Kong:** None. **S. Yun:** None. **J. Seo:** None. **G. Choi:** None. **S. Ahn:** None. **S. Shim:** None. **H. Jung:** None. **S. Kim:** None. **J. Chang:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.05/F15

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NRF-2018R1D1A1B07046189
NRF-2017R1D1A1B03034480

Title: Pain-relieving effects by downregulation of GTP cyclohydrolase I in a rat model of central neuropathic pain

Authors: *M. PARK[1,2], C. KOH[1], H. JUNG[1], J. CHANG[1];
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Abstract: Introduction: Central neuropathic pain (CNP) is one of the most common complications caused by spinal cord injury. Approximately 69% of patients with spinal cord injury suffered pain and nearly one-third of them reported excruciating chronic pain. However, to date, effective treatments targeting CNP have not yet been established. Using various pain relievers is a common treatment option but in many cases the effectiveness is still questioned. In this study, we evaluated neuroprotective and pain-reducing effects of GTP cyclohydrolase I (GCH1) in a rat model of spinal cord injury by suppressing tetrahydrobiopterin (BH4) which is a key factor for cascades of cellular and molecular microenvironment.

Methods: Sprague-Dawley rats (180g-200g) were used and neuropathic pain was induced by a spinal cord injury model. We selectively damaged spinothalamic tract using tungsten electrode and the lesion was made (0.6-0.8mm lateral to midline and 1.8-2.1mm deep) through the intra spinal cord in C6 and C7 without laminectomy. Either small hairpin RNA against GCH1 (rAAV-shGCH1) or rAAV-scrambled was injected to the dorsal part of lesion (3 ul). von Frey test based on up-and-down method was started from post-operation day 3 to evaluate the effect of rAAV-shGCH1. To identify glial activations, Iba1 expression level was measured after sacrifice.
Results: Pain alleviation effects of rAAV-shGCH1 were observed from post-operation day 3 to 14. The mechanical withdrawal threshold was 14.87 ± 0.26 in rAAV-shGCH1 group and 5.19 ± 1.87 in control group on post operation day 3 ($P < 0.05$). These pain relief effects were maintained throughout 14 days (9.73 ± 1.38 in rAAV-shGCH group and 1.64 ± 0.891, $P < 0.05$). Furthermore, the results of immunohistochemistry staining revealed microglia activations in control group.
Conclusion: We downregulated GCH1 level using rAAV-shGCH1 by selectively disrupting GCH1 mRNA to investigate its neuroprotective and pain-reducing effects in CNP. Based on our results, we confirmed the early stage protective effect of rAAV-shGCH1 and it seems downregulation of GCH1 could be a promising treatment for patients with CNP in the future.

Disclosures: **M. Park:** None. **C. Koh:** None. **H. Jung:** None. **J. Chang:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.06/F16

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: KHIDI- HI19C0060
NRF-2016M3C7A1914123
NRF-2016R1D1A3B03932649

Title: Optimizing skull penetration of focused ultrasound through analysis of energy efficiency for various skull factors

Authors: ***C. KONG**[1], J. SHIN[1,2], Y. NA[3], H. BEAK[4], J. PARK[4], W. CHANG[1], J. CHANG[1], J. CHANG[2];
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Abstract: Introduction: Focused ultrasound (FUS) has shown clinical efficacy for the treatment of Parkinson's disease, essential tremor, and obsessive-compulsive disorder. In addition to the ablative uses of FUS, current research has focused on the application of low intensity focused ultrasound to open the BBB in preclinical therapeutic studies and clinical trials. In clinics, FUS has a non-invasive merit, but also has the disadvantage that the ultrasound energy is attenuated by the skull, resulting in a difference in energy efficiency among patients. In this study, we investigated the energy efficiency for various skull factors in FUS.
Materials and Methods: Thickness and density of skull, and proportion of trabecular and cortical bone were selected as factors that could affect ultrasound energy transmittance. Sixteen 3D printed skull models were designed and fabricated to reflect the three factors. Sixteen skull phantoms were designed and fabricated to reflect the three factors using a 3D printer. The energy of each phantoms was measured using an ultrasonic sound field energy measurement system. Measuring equipment: HNC hydrophones (ONDA; HNC-1000), NTR water conditioner (ONDA; MECS), AIMS (ONDA; AST03), Waveform generator & Oscilloscope (Agilent), RF power Amplifier (E&I 240L), Transducer (Sonic concept H-115).
Results: According to the change of thickness, density, or proportion of trabecular and cortical bone, the energy passing through the phantom was also changed, and the thickness was most influenced. The peak pressure was 0.866MPa in no phantom. The peak pressure decreased from 0.084MPa (Thickness: 5mm) to 0.078MPa (Thickness: 10mm) and from 0.078MPa to 0.061MPa (Thickness: 20mm). As the thickness of the skull increases, the attenuation of the ultrasonic energy increases. The lower the ratio of cancellous bone than the cortical bone, the more the attenuation of ultrasonic energy. As the density of cancellous bone decreased, the attenuation rate of ultrasonic energy decreased.
Conclusion: We have confirmed the correlation between some of the various components of the 3D printed skull models and the ultrasound energy, and will also build up the optimal ultrasound parameters needed for clinical research through comparison with actual skulls.

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Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.07/F17

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: KHIDI- HI19C0060
NRF-2016M3C7A1914123
NRF-2016R1D1A3B03932649

Title: The modulation of activity-regulated cytoskeleton associated protein expression in the rat hippocampus using focused ultrasound

Authors: *J. SHIN[1,2], C. KONG[1], J. SIM[1,2], J. LEE[1,2], Y. SEO[1,2], Y. NA[3], W. CHANG[1], J. CHANG[1,2];
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Abstract: Introduction: Transcranial focused ultrasound (FUS) has gained attention for its potential application as a method to improve cognitive function and memory consolidation in AD mice, however the mechanism is unclear. It is known that Neuronal activity generated expression of the immediate early proteins. Arc is one of the proteins, which plays a key role in multiple forms of long-term potentiation(LTP) and depression(LTD) of synaptic transmission, adaptive functions, and homeostatic synaptic scaling such as formation of long-term memory. Here, we investigated the effect of focused ultrasound (FUS) on Arc in rat hippocampus. **Materials and Methods:** Adult male Spargue-Dawley rats (250-300 g) were used and Rats were sonicated using a single-element transducer (frequency 0.5 MHz) with microbubble. The acoustic parameters used for each sonication are: pressure amplitude 0.27 MPa, pulse length 10 ms, burst repetition frequency 1 Hz, and a duration of 120 s. Rats were sacrificed at different time points after sonication for histological and western blot analysis. **Results:** We found that the expression levels of Arc were significantly increased in the FUS-treated hippocampus following 24-hour of FUS sonication, compared to control group. Also, immediately-group showed increased expression levels after sonication, but not significantly different from control group. **Conclusion:** The present study demonstrated that increased Arc expression levels by FUS, which implies Arc may be involved in FUS-induced LTP and LTD. In further study, Arc certainly still needs to be more explored, especially on how Arc contributes to memory consolidation by FUS.

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Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.08/F18

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: DFG TR128
FTN

Title: Temporal dynamics of the emergence of cortical neuronal hyperactivity in a relapsing-remitting experimental neuroinflammation

Authors: ***T. FU**[1], E. ELLWARDT[2], D. LUCHTMAN[2], F. ZIPP[2], A. STROH[1];
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Abstract: In our previous study, using a relapsing-remitting experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis (MS), we identified a new functional subpopulation of hyperactive neurons in the cortex, exclusively in the state of remission. Our findings suggested a maladaptive, temporally delayed and cortex-wide shift of neuronal network dynamics, only indirectly linked to immune-mediated damage. In this study, we conducted longitudinal 2-photon calcium imaging in the awake behaving animal, to explore the temporal onset and stability of the hyperactive network state, with single neuron resolution. For that, we implanted a cortical chronic window, upon injection of a virus encoding for the calcium indicator GCaMP6f. This allowed for the repeated assessment of microcircuit activity of the very same neurons throughout the entire disease development of PLP-immunized relapsing-remitting SJL/J mice. In the awake mouse, we recorded spontaneous activity, and visually stimulated the animal by drifting gratings while imaging in layer II/III of mouse visual cortex. In addition, locomotion speed as an additional measure was recorded simultaneously. We could identify a time-resolved shift towards neuronal hyperactivity, observing a rather complex development of a new functional hyperactive subgroup. These findings might better define the time window for therapeutic intervention, aiming at re-balancing dysregulated cortical networks.

Disclosures: **T. Fu:** None. **E. Ellwardt:** None. **D. Luchtman:** None. **F. Zipp:** None. **A. Stroh:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.09/F19

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Scleroseforeningen
Odense University Hospital
MERCK
Sanofi-Genzyme
Jascha Fonden
Direktør Ejnar Jonasson kaldet Johnsen og hustrus mindelegat

Title: Molecular signatures of different lesions types in the white matter brain of patients with progressive multiple sclerosis

Authors: *M. L. ELKJAER[1], T. FRISCH[1], R. REYNOLDS[2], T. KACPROWSKI[3], T. A. KRUSE[1], M. THOMASSEN[1], J. BAUMBACH[3], Z. ILLES[1];
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Abstract: Hypothesis: Active inflammatory demyelinating lesions in the white matter (WM) of MS patients can be remyelinated or develop into inactive lesions. In the progressive phase, chronic active lesions become prominent in the WM. To investigate mechanisms behind lesion evolution and fate, we examined the transcriptome in normal-appearing WM (NAWM), active, inactive, remyelinating, and chronic active lesions from progressive MS brains and control WM. **Methods:** Next generation RNA sequencing was performed on 73 lesions and 25 control tissue. To investigate unique transcriptional changes in different lesion types, we (i) examined protein-protein interactions in each lesion type by *de novo* network enrichment of differentially expressed genes (DEG); (ii) determined lesion-specific pathways based on DEGs only in one lesion type; (iii) identified signatures of significant DEGs different between at least two lesion types; (iv) validated expression and cellular source by immunohistochemistry (IHC), immunofluorescence (IF) and RNAscope. We also created a user-friendly web interface (MS Atlas) which allows for interactively analyzing the MS brain lesion types. **Results:** Out of 18722 expressed genes, 4223 were DEGs in MS compared to control. *CD26/DPP4* was among the six upregulated DEGs in NAWM expressed by microglia. We identified two clusters of 62 DEGs that separated chronic active from all other lesion types by an inverse regulation pattern. The gene of an emerging biomarker, *CHI3L1* (chitinase-3-like protein-1) was such unique upregulated DEG in chronic active lesions: combined IHC and RNAscope revealed expression on astrocytes in the rim of chronic active lesions. *De novo* network enrichment of DEGs with inverse regulation in chronic active *versus* remyelinating lesions identified TGFβ-R2 as a central hub for remyelinating lesions: IHC and RNAscope showed TGFβ-R2 expression by astrocytes in remyelinating lesions. **Discussion:** The transcriptome signature of chronic active lesions was different from all other lesion types. Microglial CD26 and astrocytic CHI3L1 may be key molecules in early vs. chronic active lesion evolution. TGFβ-R2 expressed by astrocytes in remyelinating lesions may be important in repair. The compendium of mechanistic MS lesion type profiles in MS Atlas is a novel interactive tool to fuel multiple sclerosis research and a new basis for MS treatment hunt.

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Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.10/F20

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Tisch MS Research Center of New York (Private Funds)

Title: Metabotropic glutamate receptors are implicated in cerebellar dysfunction in multiple sclerosis

Authors: *C. ARNDTSEN, N. FAVRET, A. IACOANGELI, S. A. SADIQ;
Tisch MS Res. Ctr. of New York, New York, NY

Abstract: Cerebellar dysfunction (CD), a common symptom of multiple sclerosis (MS), is difficult to treat since disease-modifying therapies do not attenuate its severity. Moreover, the molecular mechanisms of CD have not been fully elucidated mainly because the most common murine MS model, experimental autoimmune encephalomyelitis, does not adequately replicate clinical features of CD. Our goal was to design a CD model by injecting cerebrospinal fluid (CSF) of MS patients with CD into the mouse cisterna magna (CM). We injected mice with 10 µl of saline solution, 10 µl of concentrated (20X) CSF from MS patients with CD, or 10 µl of concentrated (20X) CSF from MS patients without CD. We injected CSF samples from seven patients per treatment group and performed experiments randomized, blinded, and in triplicate. The motor coordination of the mice was assessed with a rotarod test administered hourly for 8 hours after injection and again at 24 hours. Rotarod performances were analyzed with a two-way ANOVA and a post-hoc Bonferroni test. Motor coordination was significantly impaired in mice injected with CSF from MS patients with CD compared to the two other groups at hours 1 and 2 post-injection. These exploratory results suggest that our mouse model of CD is functional and can be utilized to understand mechanisms and treatments. Using our CD mouse model, we investigated whether group 1 metabotropic glutamate receptors (mGluR) are involved in CD since mGluR1 knockout mice display motor deficits and impaired motor learning. We co-injected the CM of mice with 250 µm of -3,5-Dihydroxyphenylglycine (DHPG, 1 µg), a selective agonist for mGluR1 and mGluR5, and concentrated CSF from MS patients with CD. In the control group, mice were injected with concentrated CSF from MS patients with CD and 1 µl of saline solution. CSF samples from five different MS patients with CD have been tested in triplicate. We observed that motor coordination was significantly ameliorated in mice injected with CSF plus DHPG compared to the control group. In conclusion, we developed a novel murine model to study cerebellar dysfunction in MS. Moreover, our findings suggest that group 1 mGluRs are implicated in CD and should be further investigated.

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Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

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Program #/Poster #: 048.11/F21

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: The National Research Foundation of Korea (NRF): NRF-2018R1C1B6008884
The National Research Foundation of Korea (NRF): NRF-2018M3A9E8066249

Title: Myelin oligodendrocyte glycoprotein antidodies in patients with CNS inflammatory demyelinating disease and recent clinical attacks

Authors: *S. KIM[1,2], E.-J. LEE[1,2], L. CHOI[1];
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Abstract: Melin oligodendrocyte glycoprotein (MOG) is a component of myelin proteins in the central nervous system (CNS). Recent identification of anti-MOG antibody (MOG-IgG) has broadened the diversity of inflammatory demyelinating diseases. Although MOG-IgG has been implicated pathogenically with certain phenotypes of CNS demyelinating diseases, it is plausible that demyelination and subsequent tissue damage may secondarily promote the development of MOG-IgG. Here we report the seroprevalence of MOG-IgG in CNS demyelinating diseases according to the presence of recent clinical attacks within 1 month. Between June 2018 and February 2019, all patients with CNS inflammatory demyelinating disease in our center underwent cell-based assays for anti-aquaporin-4 antibodies (AQP4-IgG) and MOG-IgG antibodies; a total of 309 patients were enrolled. Of them, 34 patients had recent clinical attacks within 1 month prior to the serum sampling. Median (quartiles) interval from the last attack to the sampling was 9 (4.8─19.3) days in the recent attack group, while it was 849 (232─2514) days in the remote attack group. As for seroprevalence, the MOG-IgG was more frequently found in the recent attack group (20.6% [7/34] vs. 4.1% [11/271], p=0.002). In the recent attack group, MOG-IgG was positive in 50.0% (4/8) of patients with AQP4-IgG-negative optic neuritis, 50.0% (2/4) of those with other CNS demyelinating diseases, and 20.0% (1/5) of those with AQP4-IgG-negative neuromyelitis optica spectrum disorder (NMOSD). In the remote attack group, the seroprevalence of MOG-IgG was only 18.8% (3/16) in AQP4-IgG-negative optic neuritis, 7.1% (1/14) in AQP4-IgG-negative NMOSD, 6.7% (1/15) in other CNS demyelinating disease, 5.3% (3/57) in AQP4-IgG-negative myelitis, and 2.7% (3/112) in MS. Notably, all the MS patients with MOG-IgG had the last attack more than 6 months prior to the serum sampling. In summary, regardless of recent clinical attacks, AQP4-IgG-negative optic neuritis was the most common phenotype that reveals MOG-IgG in our population. However, the MOG-IgG seroprevalence and its associated diseases were distinct according to recent attacks, suggesting

that the MOG-IgG seropositivity may be more specific during the acute phase of the demyelinating diseases.

Disclosures: S. Kim: None. **E. Lee:** None. **L. Choi:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.12/F22

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: This study was funded by Alkermes (Waltham, MA, USA); medical writing support was provided by Excel Scientific Solutions (Southport, CT, USA) and funded by Biogen.

Title: The diroximel fumarate (DRF) metabolite, 2-hydroxyethyl succinimide (HES), demonstrated no effect on DRF efficacy in an animal model of multiple sclerosis and *in vitro* pharmacological assessments

Authors: *M. PALTE[1], N. PENNER[1], M. TURNER[3], J. HANNA[2], L. DAHM[3];
[1]Biogen, Cambridge, MA; [2]Biogen, Maidenhead, United Kingdom; [3]Alkermes, Waltham, MA

Abstract: Diroximel fumarate (DRF; ALKS 8700; BIIB098) is a novel oral fumarate in development for patients with relapsing forms of multiple sclerosis (MS). Upon oral administration, DRF is rapidly metabolized to the major pharmacologically active metabolite, monomethyl fumarate (MMF), which is also produced by dimethyl fumarate (DMF), an approved treatment for patients with relapsing forms of MS. An additional major metabolite, 2-hydroxyethyl succinimide (HES), is produced upon metabolization of DRF. Any potential biological activity of HES and theoretical impact to the DRF efficacy profile was assessed in a Lewis rat model of MS and in two *in vitro* activity assays: the CEREP panel of 133 binding and enzyme/uptake assays and the DiscoverX BioMAP Diversity Plus panel of 148 protein biomarker and functional activities in a diverse range of cell types that model aspects of vascular, immune, inflammation, lung, skin, and tissue biology. A Lewis rat model of experimental autoimmune encephalomyelitis (EAE) was used to compare *in vivo* efficacy of orally administered DMF and DRF at matched MMF area under the curve exposure levels in animals (12/group) immunized with myelin basic protein derived peptide to induce EAE symptoms. Administration of DRF in the EAE model resulted in systemic HES exposure that was 12x >MMF and 3x >clinically observed exposure with DRF 462 mg twice-daily dose. Cohorts treated with DMF or DRF demonstrated a delay in disease initiation and a reduction in overall disease severity compared with the vehicle-treated cohort. No significant differences were observed between the administered compounds, confirming similar activities for DRF and DMF

and no interference of the HES metabolite on DRF efficacy. Furthermore, HES 300 µM (4x >clinically observed exposure) demonstrated no pharmacological activity in the CEREP panel, and HES 450 µM (6x >clinically observed exposure) demonstrated no effect in the DiscoverX BioMAP Diversity Plus panel. HES + MMF (450 µM each) had a profile similar to MMF alone. Together, these data demonstrate that HES is pharmacologically inactive and has no interfering effect on DRF activity, supporting further development of DRF as a potential new treatment option for patients with relapsing forms of MS.

Disclosures: **M. Palte:** A. Employment/Salary (full or part-time):; Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **N. Penner:** A. Employment/Salary (full or part-time):; Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **M. Turner:** A. Employment/Salary (full or part-time):; Alkermes. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkermes. **J. Hanna:** A. Employment/Salary (full or part-time):; Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biogen. **L. Dahm:** A. Employment/Salary (full or part-time):; Alkermes. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkermes.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.13/F23

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NS-R37041435
National MS Society Collaborative Center Grant
Medical Scientist Training Program at Johns Hopkins University

Title: Complement component 3 knock-out rescue the RGC loss in experimental autoimmune encephalomyelitis mouse model

Authors: ***J. JIN**[1], C. J. KERSBERGEN[2], M. D. SMITH[1], K. WHARTENBY[1], P. A. CALABRESI[1,2];
[1]Neurol., [2]Solomon Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Multiple sclerosis (MS) is a neuroimmunological disorder characterized by demyelination, inflammation and neurodegeneration of the central nervous system (CNS),

including the retina and optic nerve. Many patients have vision impairment and loss of visual quality of life which is associated with neuronal and axonal degeneration. Several studies have implicated the classical complement cascade, especially complement component 3 (C3) expressed by neurotoxic astrocytes, in mediating neurodegeneration. Finally, C3 gene variants have been associated with more rapid rates of retinal ganglion cell layer thinning in people with MS using optical coherence tomography. Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model of CNS autoimmunity and has been successfully used to study mechanisms of disease involved in MS. Here we used a MOG_{35-55} induced EAE mouse model to explore the role of C3 in the pathogenesis of retinal neurodegeneration following optic neuritis. EAE was induced both in WT and C3 knock out (C3-/-) mice. Mouse eyeballs and optic nerves were collected at early/peak and late stage of the disease. Pathology studies were performed on retinas and optic nerve. WT and C3-/- retinas had no loss of RGCs at peak disease, but the late WT animals had significantly more RGC loss than C3-/- at the late time point. Interestingly, this was associated with preservation of the post-synaptic protein PSD95 in C3-/- mice but not WT. Immunohistochemical analyses of the optic nerves showed no difference in the inflammatory infiltrate, extent of demyelination, or activation of microglia and astrocytes, at both early and late stage. In human MS eyeballs, we also found moderate to severe RGC loss compared with non-neurological controls. Microglia and C3+/GFAP+ astrocyte activation was observed in the inner retina in MS tissues. Our findings suggest that C3 may play a role in the pathology of RGC loss in the mouse EAE model and possibly some patients with MS. Further studies of the cellular source of C3 and mechanisms by which C3 may be associated with neuronal degeneration may help to elucidate the pathogenesis of RGC loss following optic neuritis and facilitate the rational design of neuroprotective agents.

Disclosures: **J. Jin:** None. **C.J. Kersbergen:** None. **M.D. Smith:** None. **K. Whartenby:** None. **P.A. Calabresi:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.14/F24

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: The National Research Foundation of Korea (NRF): NRF-2018R1C1B6008884
The National Research Foundation of Korea (NRF): NRF-2018M3A9E8066249

Title: Myelin oligodendrocyte glycoprotein antibodies in patients with CNS inflammatory demyelinating disease and recent clinical attacks

Authors: ***S.-M. KIM**[1,2], L. CHOI[1], S. LEE[1], H.-W. KIM[1], H. KIM[1], S. KIM[1], Y.-M. LIM[1], K.-K. KIM[1], E.-J. LEE[1,2];

[1]Dept. of Neurol., Asan Med. Center, Univ. of Ulsan, Col. of Med., Seoul, Korea, Republic of; [2]Asan Med. Inst. of Convergence Sci. and Technol., Seoul, Korea, Republic of

Abstract: Myelin oligodendrocyte glycoprotein (MOG) is a component of myelin proteins in the central nervous system (CNS). Recent identification of anti-MOG antibody (MOG-IgG) has broadened the diversity of inflammatory demyelinating diseases. Although MOG-IgG has been implicated pathogenically with certain phenotypes of CNS demyelinating diseases, it is plausible that demyelination and subsequent tissue damage may secondarily promote the development of MOG-IgG. Here we report the seroprevalence of MOG-IgG in CNS demyelinating diseases according to the presence of recent clinical attacks within 1 month. Between June 2018 and February 2019, all patients with CNS inflammatory demyelinating disease in our center underwent cell-based assays for anti-aquaporin-4 antibodies (AQP4-IgG) and MOG-IgG antibodies; a total of 309 patients were enrolled. Of them, 34 patients had recent clinical attacks within 1 month prior to the serum sampling. Median (quartiles) interval from the last attack to the sampling was 9 (4.8─19.3) days in the recent attack group, while it was 849 (232─2514) days in the remote attack group. As for seroprevalence, the MOG-IgG was more frequently found in the recent attack group (20.6% [7/34] vs. 4.1% [11/271], p=0.002). In the recent attack group, MOG-IgG was positive in 50.0% (4/8) of patients with AQP4-IgG-negative optic neuritis, 50.0% (2/4) of those with other CNS demyelinating diseases, and 20.0% (1/5) of those with AQP4-IgG-negative neuromyelitis optica spectrum disorder (NMOSD). In the remote attack group, the seroprevalence of MOG-IgG was only 18.8% (3/16) in AQP4-IgG-negative optic neuritis, 7.1% (1/14) in AQP4-IgG-negative NMOSD, 6.7% (1/15) in other CNS demyelinating disease, 5.3% (3/57) in AQP4-IgG-negative myelitis, and 2.7% (3/112) in MS. Notably, all the MS patients with MOG-IgG had the last attack more than 6 months prior to the serum sampling. In summary, regardless of recent clinical attacks, AQP4-IgG-negative optic neuritis was the most common phenotype that reveals MOG-IgG in our population. However, the MOG-IgG seroprevalence and its associated diseases were distinct according to recent attacks, suggesting that the MOG-IgG seropositivity may be more specific during the acute phase of the demyelinating diseases.

Disclosures: S. Kim: None. **E. Lee:** None. **L. Choi:** None. **S. Lee:** None. **H. Kim:** None. **H. Kim:** None. **S. Kim:** None. **Y. Lim:** None. **K. Kim:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.15/F25

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: A novel treatment strategy for multiple sclerosis

Authors: *F. MUBARIZ[1], M. ARVAS[1], V. GERZANICH[2], C. T. BEVER, Jr.[3], T. K. MAKAR[4];
[1]Neurol., [2]Neurosurg., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Office of Res. and Develop., DVA, Washington, DC; [4]Neurol., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Currently approved therapies for Multiple sclerosis (MS), which target the adaptive immune system have not been shown to prevent disability or progressive stage of the disease. Studies on multiple sclerosis (MS), and its animal model experimental autoimmune encephalomyelitis (EAE), have suggested that chemokines might be key players in disease development. Astrocytes are important in innate immunity and participate actively and variably at different stages of the pathologic process. There are ten approved “disease modifying” therapies for MS, none of which specifically target astrocytes. We have shown that glibenclamide (GLB) treatment has neuroprotective effects mediated through inhibition of the transient receptor potential melastatin 4 (Trpm4) / sulfonylurea receptor 1 (Sur1) complex in brain injury conditions we tested it in EAE; and found that it reduced clinical and pathological severity of disease. In addition, chronic treatment of GLB also down regulates clinical and pathological severity by upregulating remyelination and axonal protection. However, the stimulation of remyelination and axonal protection of GLB mechanism is unknown. It is identified that endothelin-1 (ET-1), which is expressed at high levels by reactive astrocytes in multiple sclerosis (MS) lesions, limits repair by delaying oligodendrocyte progenitor cell (OPC) maturation. We have shown that ET-1 acts selectively through ET-1 receptor B on astrocytes and indirectly inhibits remyelination. These results demonstrate that targeting specific pathways in reactive astrocytes represents a promising therapeutic target in MS.

Disclosures: F. Mubariz: None. **M. Arvas:** None. **V. Gerzanich:** None. **C.T. Bever:** None. **T.K. Makar:** None.

Poster

048. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 048.16/F26

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Expression profile of neural, trophic, and immunomodulatory genes in multiple sclerosis donor-derived mesenchymal stem cell-neural progenitors

Authors: J. GREENWALD, S. A. SADIQ, *V. K. HARRIS;
Tisch MS Res. Ctr. of New York, New York, NY

Abstract: Multiple sclerosis (MS) is an autoimmune-mediated demyelinating disease of the CNS. Patients with progressive MS experience a steady worsening of neurologic function

attributed to chronic demyelination and axonal loss. A novel regenerative therapy utilizing autologous mesenchymal stem cell-derived neural progenitors (MSC-NP) is currently under clinical investigation in patients with progressive MS. MSC-NPs promote neural repair through the paracrine release of trophic and immuno-modulatory factors. Recent results from a phase I trial demonstrated reversal of established disability after repeated intrathecal MSC-NP injections. A phase II randomized double-blind placebo-controlled trial is underway to confirm the efficacy of this approach. As this autologous cell therapy moves into clinical use, there is a need to better define and characterize MSC-NPs in order to better understand the mechanisms underlying therapeutic potency. The objective of this study was to define the transcriptional profile of MSC-NPs from secondary progressive MS (SPMS), primary progressive MS (PPMS), and non-MS donors in order to better understand their functional characteristics and therapeutic potential in multiple sclerosis. MSCs were derived from sternal bone marrow of MS patients (SPMS, n=4; PPMS, n=4) as part of an IRB-approved study protocol (Western IRB). MSCs from non-MS donors (n=2) were isolated from a stroke patient, and from a healthy donor bone marrow aspirate. MSCs were expanded for up to 4 passages in growth medium containing 5% human platelet lysate, then transferred to neural progenitor medium containing EGF/bFGF to generate MSC-NPs. Population doubling time (PDT) of MSCs was determined by cell counting. RNA isolated from MSC and MSC-NP cells was analyzed for gene expression differences by RNA sequencing and validated by quantitative PCR. Donor characteristics had no impact on cell growth rate or the yield of MSC-NPs generated. Transcriptional profiling of MSC/MSC-NP pairs confirmed the upregulation of neural genes such as Nestin, and the downregulation of mesodermal genes such as Thy-1 and Acta2, thus affirming MSC-NP identity. In addition, gene candidates that mediate trophic/immunoregulatory mechanisms of action of MSC-NPs were identified, including HGF and TGF-ß. Characterization of the transcriptional profile of MSC-NPs has revealed potential pathways that mediate therapeutic mechanisms of this novel cell therapy in MS. These studies form the basis of marker-based potency assays that may be used to better predict the therapeutic efficacy of individual batches of autologous MSC-NPs administered to patients during clinical trials.

Disclosures: V.K. Harris: None. **J. Greenwald:** None. **S.A. Sadiq:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.01/F27

Topic: C.08. Ischemia

Support: National Research Foundation of Korea (NRF), 2017R1A2B4007301

Title: Phosphorylation of Fbxw7 by Cdk5 causes decreased stability of Fbxw7 in glutamate mediated excitotoxicity

Authors: *Y.-J. OH, **Y. KO**;
Dept. of Systems Biol., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Fbxw7 is a component of the SCF^{Fbxw7} E3 ligase complex that regulates cell division and growth. So far, many of Fbxw7-related research have focused on cancer metabolism. However, it has been also reported that Fbxw7 regulates brain development and differentiation. Cyclin-dependent kinase 5 (Cdk5) is a brain-specific serine/threonine protein kinase that regulates brain development and neurodegeneration. In physiologic condition, Cdk5 is activated by its activator proteins, p35 and its physiological activity is appropriately regulated. However, in pathologic condition, Cdk5 is hyper-activated by p25 that is generated from cleavage of p35, resulting from calpain activation. Unlike p35, the generation of p25 is culpable for the aberrant hyper-activation of Cdk5, causing neurodegeneration. Therefore, decreased Cdk5/p25 activity was one of target for alleviate neurodegeneration. Here, we discovered that F-box/WD repeat-containing protein 7 (Fbxw7) is a new substrate of Cdk5 and hyper-activation of Cdk5 was eventually linked to decreased stability of Fbxw7. We also observed decreased stability of Fbxw7 in various calpain-activating conditions: primary cultures of cortical neurons challenged with glutamate and rat brains received a middle cerebral artery occlusion (MCAO). Decreased levels of Fbxw7 led to increased levels of transcription factor AP-1 (c-Jun) that is a known substrate of Fbxw7, which could possibly lead to accelerated cell death by c-Jun-mediated apoptosis. Thus, our data reveal a novel Cdk5-Fbxw7-c-Jun death pathway and raise the possibility that maintenance of Fbxw7 may comprise a critical point of neuroprotection.

Disclosures: **Y. Oh:** None. **Y. Ko:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.02/F28

Topic: C.08. Ischemia

Title: Transient cerebral ischemia induces NADPH oxidase-mediated oxidative damage to proteins in the postsynaptic density

Authors: ***K. MUROTOMI**[1], N. TAKAGI[2], K. TANONAKA[2];
[1]Biomed. Res. Institute, Mol. Neurophysiol. Res. Group, Advanced Industrial Sci. and Technol. (AIST), Ibaraki, Japan; [2]Tokyo Univ. Phar & Life Sci., Hachioji, Japan

Abstract: Superoxide is produced after cerebral ischemia and reperfusion, and the excessive superoxide induces brain damage. NADPH oxidase is a source of superoxide in the central nervous system. NADPH oxidase is localized near the postsynaptic site in neurons. However, the role of NADPH oxidase in the synaptic apparatus after cerebral ischemia and reperfusion remains to determined. To examine this issue, we sought to determine the pathophysiological role of NADPH oxidase to postsynaptic density (PSD) proteins, which were isolated from rats subjected to transient focal cerebral ischemia and reperfusion. The amounts of carbonylated proteins in the PSD were increased after transient focal cerebral ischemia. This change was accompanied by an increase in the level of NADPH oxidase subunits. The increase in the level of carbonyl proteins in the PSD after cerebral ischemia and reperfusion was attenuated by the administration of apocynin, an NADPH oxidase inhibitor. Furthermore, we demonstrated that the decreases seen in the amounts of PSD-associated proteins, such as neuroligin, N-cadherin, and SAP102, after transient cerebral ischemia in the PSD were attenuated by treatment with apocynin. Therefore, activation of NADPH oxidase in the PSD after cerebral ischemia and reperfusion may be related to synaptic dysfunctions and subsequent development of cell injury after cerebral ischemia and reperfusion.

Disclosures: K. Murotomi: None. **N. Takagi:** None. **K. Tanonaka:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.03/F29

Topic: C.08. Ischemia

Title: Endothelium-targeted deletion of microRNA-15a/16-1 ameliorates blood-brain barrier dysfunction in ischemic stroke

Authors: F. MA[1], P. SUN[1], X. ZHANG[1], M. HAMBLIN[2], *K. YIN[1];
[1]Univ. of Pittsburgh Sch. of Med., Puittsburgh, PA; [2]Tulane Univ. Sch. of Med., Dept. of Pharmacol., New Orleans, LA

Abstract: The Blood-Brain Barrier (BBB) maintains a stable brain microenvironment and the breakdown of BBB integrity has been proven to initiate a devastating cascade of events and eventually cause neuronal functional impairments. It is important to identify mechanisms by which the BBB disruption can be reduced under ischemic stroke conditions. MicroRNAs function as a novel class of small noncoding RNAs that negatively modulate protein expression. We and others have recently revealed the involvement of miR-15a/16-1 in the pathogenesis of ischemic brain injury. However, the functional significance and molecular mechanisms of the miR-15a/16-1 cluster in regulating BBB integrity are poorly understood in ischemic stroke. In this study, endothelial cell-targeted deletion of 15a/16-1 (EC-miR15a/16-1 cKO) mice and wild-

type (WT) controls were subjected to 1h Middle Cerebral Artery occlusion (MCAo) followed by 1/3-d reperfusion, and the regulatory role of miR-15a/16-1 cluster in post-stroke BBB integrity was investigated. Compared with WT controls, genetic deletion of miR-15a/16-1 in endothelium led to less BBB leakage and water content, reduced the infiltration of peripheral macrophages and neutrophils in stroke mice. Moreover, EC-selective deletion of miR-15a/16-1 reduced the number of M1-type microglia/macrophages in the peri-infarct area without affecting the M2-type microglia/macrophages in ischemic mice. Mechanistically, EC-miR15a/16-1 cKO mice exhibited significantly enhanced claudin-5 expression in cKO mouse ischemic brain area compared to WT controls. In our in vitro studies, lentivirus-mediated gain- or loss-of miR-15a/16-1 function was performed in mouse primary brain microvascular endothelial cells (mBMECs). Lentiviral silencing of miR-15a/16-1 cluster significantly increased claudin-5 expression in mBMECs after exposure to Oxygen-Glucose Deprivation (OGD) and diminished the OGD-induced disintegration of the BBB in mBMECs/Astrocytes co-culture model, evidenced by significantly increased transendothelial electrical resistance (TEER) and reduced paracellular permeability. Moreover, the miR-15a/16-1 cluster was found to repress claudin-5 function by directly binding to the 3'-untranslated region (3'-UTR) of mouse claudin-5 gene. These findings suggested that the miR-15a/16-1 cluster functions as a novel negative regulator in BBB pathologies after ischemic stroke. Elucidating the molecular mechanisms of miR-15a/16-1-mediated BBB dysfunction may lead us to discover novel pharmaceutical target for the development of effective therapies against ischemic stroke.

Disclosures: **F. Ma:** None. **P. Sun:** None. **X. Zhang:** None. **M. Hamblin:** None. **K. Yin:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.04/F30

Topic: C.08. Ischemia

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Science and Technology Planning Project of Wuhan (No. 2017060201010202) to FP

Title: Neuroglobin boosts axon regeneration during ischemic reperfusion via p38 binding and activation depending on oxygen signal

Authors: *C. LI[1], X. XIONG[1], F. PAN[2], R. CHEN[1], D. HU[1], X. QIU[1], X. XIE[3], B. TIAN[4], X. CHEN[1];
[1]Dept. of Pathophysiology, Sch. of Basic Medicine, Tongji Med. College; Inst. of Brain Research; Key Lab. of Neurolog. Diseases, Ministry of Education; Huazhong Univ. of Sci. and Technol., Wuhan, China; [2]Dept. of Urology, Union Hospital, Huazhong Univ. of Sci. and Technol., Wuhan, China; [3]Dept. of Pathology, Jingzhou Central Hospital, The Second Clin. Med. College, Yangtze Univ., Jingzhou, China; [4]Dept. of Neurobiology, Sch. of Basic Medicine, Tongji Med. College, Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: Cerebral ischemia causes severe cell death or injury including axon breakdown or retraction in the brain. Axonregeneration is crucial for the functional recovery of injured neurons or brains after ischemia/reperfusion (I/R); however, this process has been proved extremely difficult in adult brains and there is still no effective therapy for it. Here we reported that neuroglobin (Ngb), a novel oxygen-binding or sensor protein existing predominantly in neurons or brains, functions as a driving factor for axon regeneration during I/R. Ngb was upregulated and accumulated in growth cones of ischemic neurons in primary cultures, rat, and human brains, correlating positively to the elevation of axon-regeneration markers GAP43, neurofilament-200, and Tau-1. Ngb overexpression promoted while Ngb knockdown suppressed axon regeneration as well as GAP43 expression in neurons during oxygen-glucose deprivation/reoxygenation (OGD/Re). By using specific pharmacological inhibitors, we identified p38 MAPK as the major downstream player of Ngb-induced axon regeneration during OGD/Re. Mechanistically, Ngb directly bound to and activated p38 in neurons upon OGD/Re. Serial truncation and point mutation of Ngb revealed that the 7-105 aa fragment of Ngb was required and the oxygen-binding site (His64) of Ngb was the major regulatory site for its p38interaction/activation. Finally, administration of exogenous TAT-Ngb peptides significantly enhanced axonregeneration in cultured neurons upon OGD/Re. Taken together, Ngb promotes axon regeneration via O2-Ngb-p38-GAP43 signaling during I/R. This novel mechanism suggests potential therapeutic applications of Ngb for ischemicstroke and other related axonopathy.

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Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.05/F31

Topic: C.08. Ischemia

Support: NIH Grant (NS097000)
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American Heart Association (16BGIA27250263)

Title: Genetic variation contributes to gene expression response in blood of ischemic stroke patients: An eQTL study

Authors: H. AMINI[1], N. SHROFF[1], P. P. SITORUS[1], P. CARMONA-MORA[1], X. ZHAN[1], B. STAMOVA[1], G. C. JICKLING[2,1], B. P. ANDER[1], *F. R. SHARP[1];
[1]Univ. of California Davis, Sacramento, CA; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Several genetic variants and single nucleotide polymorphisms (SNPs) are associated with ischemic stroke (IS). It is further known that specific gene expression patterns drastically change after ischemic stroke. One of the main biomolecular mechanisms that contribute to the injury response is variation in gene activation and expression. However, SNPs that are associated with the magnitude of change in blood gene expression after ischemic stroke are largely unknown. Thus, we study the association of common genetic variants with changes in mRNA expression levels (i.e. expression quantitative trait loci; eQTL) in blood after ischemic stroke. RNA and DNA were isolated from blood samples collected from 168 ischemic stroke patients and 183 vascular risk factor controls (VRFC). Gene expression of all protein-coding transcripts was quantified by Affymetrix HTA 2.0 microarrays and variants assessed by Axiom Biobank Genotyping microarrays. In order to identify ischemic stroke diagnosis-dependent eQTL, a linear model with a genotype (SNP) × diagnosis (IS and VRFC) interaction term was fit for each SNP-gene pair.

Our eQTL interaction analysis found significant genotype × diagnosis interaction term for eight SNP-gene pairs as cis-eQTL and 33 SNP-gene pairs as trans-eQTL. The strongest cis-eQTL, rs78046578, was related to expression of C-X-C Motif Chemokine Ligand 10 (*CXCL10*), a member of the CXC chemokine family that binds to the CXCR3 receptor and is involved in chemotaxis, induction of apoptosis, regulation of cell growth and chemokine-mediated angiostatic effects. In addition, *CXCL10* is upregulated in human post-mortem ischemic stroke brain and involved in the BBB breakdown following stroke. The trans-eQTL, rs148791848, was associated with expression of *ANOS1*. *ANOS1* gene encodes protein anosmin-1 that is key in developmental processes and implicated in neural cell adhesion and important in axonal migration.

This study describes the importance of the role of genetic variants in the expression of genes that relate to post-ischemic stroke injury and/or recovery. Further work examining these relationships will help determine modifiable treatment strategies to improve stroke outcome.

Disclosures: H. Amini: None. **N. Shroff:** None. **P.P. Sitorus:** None. **P. Carmona-Mora:** None. **X. Zhan:** None. **B. Stamova:** None. **G.C. Jickling:** None. **B.P. Ander:** None. **F.R. Sharp:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.06/F32

Topic: C.08. Ischemia

Support: Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, Amarillo, TX

Title: Inhibition of long chain fatty acyl CoA synthetase modulates inducible nitric oxide synthase expression

Authors: *M. ALI, M. WEIS;
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Abstract: Stroke kills about 140,000 Americans each year and is the leading cause of long-term disability. Eighty-seven percent of all stroke is ischemic stroke. Nitric oxide (NO), synthesized by nitric oxide synthase (NOS), has a dual role in the ischemic condition. Endothelial nitric oxide synthase (eNOS) derived NO regulates vascular tone in a way that it considered neuroprotective. In contrast, inducible nitric oxide synthase (iNOS) expression increases from 12 hours after the onset of ischemic stroke and can last for 1 week. When iNOS is induced, it can produce 100 to 1000 times more NO than eNOS, concentrations that are neurotoxic, leading to neuronal cell death. Our lab has published that Triacsin C, a fungal metabolite which inhibits long chain fatty acyl CoA synthetase (ACSL), decreases eNOS palmitoylation and increases eNOS derived NO without upregulating eNOS transcription in cultured human coronary endothelial cells. In addition, Triacsin C reduces the infarct volume and inhibits iNOS expression in the mouse middle cerebral artery occlusion (MCAO) model of stroke. In the current study, we wanted to see whether iNOS expression is modulated by ACSL activity. Cells were stimulated with cytokine mix (TNFα, 60 ng/mL; IL-1β, 2 ng/mL; IFNγ, 100 units/mL) in the presence or absence of 5μM Triacsin C for 24 hours. In our qPCR experiment, cytomix stimulated iNOS expression in the presence of Triacsin C was 72.9 1.96 % of cytomix group ($p = 0.0001$, $n = 3$) in C6 astrocytoma cells and 45.6 6.7 % of cytomix group ($p = 0.0001$, $n = 6$) in bEnd.3 cells. No change has been observed in nNOS and eNOS mRNA expression in any group compared to the control in C6 astrocytoma cells and bEnd.3 cells respectively. We also observed that ACSL inhibition with Triacsin C reduced cytokine-stimulated iNOS protein expression in bEnd.3 cells from our western blot analysis. Total nitric oxide synthase activity was measured by conversion of [14C] arginine to [14C] citrulline in the presence or absence of calcium in C6 cells. Cytomix stimulated iNOS activity in the presence of Triacsin C was 79.95 6.04 % ($p =0.0423$, $n = 3$) and 81.02 4.29 % of cytomix group ($p = 0.0095$, $n = 3$) in the presence or absence of calcium respectively. Our data confirm that iNOS expression is modulated by ACSL activity, and support

our hypothesis that the Triacsin C effect on stroke infarct volume is related to the suppression of iNOS expression.

Disclosures: **M. Ali:** None. **M. Weis:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.07/F33

Topic: C.08. Ischemia

Support: NIH Grant NS104349
NIH Grant SC3GM122593
NIH Grant S21MD000101

Title: β-estradiol protects against acidosis-mediated and ischemic neuronal injury by promoting ASIC1a protein degradation

Authors: *T.-D. LENG, R.-P. ZHOU, T. YANG, Z.-G. XIONG;
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Abstract: Gender differences in the incidence and outcome of stroke have been well documented. The severity of stroke in women is, in general, significantly lower than that in men, which is mediated, at least in part, by the protective effects of β-estradiol. However, the detailed mechanisms are still elusive. Recent studies have demonstrated that activation of acid-sensing ion channel 1a (ASIC1a) by tissue acidosis, a common feature of brain ischemia, plays an important role in ischemic brain injury. In the present study, we assessed the effects of β-estradiol on acidosis-mediated and ischemic neuronal injury both in vitro and in vivo and explored the involvement of ASIC1a and underlying mechanism. We demonstrated that treatment of neuronal cells with β-estradiol in vitro decreased acidosis-induced cytotoxicity. ASIC currents and acid-induced elevation of intracellular Ca^{2+} were all attenuated by β-estradiol. In addition, we showed that β-estradiol treatment reduced ASIC1a protein expression which was mediated by an increased protein degradation and that estrogen receptor α was involved. Finally, we showed that the level of ASIC1a protein expression in brain tissues and the degree of neuroprotection by ASIC1a blockade were lower in female mice, which could be elevated by ovariectomy.
These results indicate that β-estradiol can protect neurons against acidosis-mediated neurotoxicity and ischemic brain injury by suppressing ASIC1a protein expression and channel function.

Disclosures: **T. Leng:** None. **R. Zhou:** None. **T. Yang:** None. **Z. Xiong:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.08/F34

Topic: C.08. Ischemia

Support: Conacyt Grant 252702
Conacyt Grant 222720

Title: Fenofibrate PPARα agonist reduces oxidative stress in cardiomyocytes exposed to high glucose and hypoxia reperfusion

Authors: *F. CORTÉS LÓPEZ[1], M. D. IBARRA- LARA[2], M. A. SÁNCHEZ- MENDOZA[2], E. SORIA-CASTRO[3], M. SÁNCHEZ- AGUILAR[2], L. DEL VALLE- MONDRAGÓN[2], L. G. CERVANTES- PÉREZ[2], V. RAMIREZ- GONZALEZ[5], V. CASTREJÓN[4], A. SÁNCHEZ-LÓPEZ[1], G. PASTELÍN- HERNÁNDEZ[2], D. CENTURIÓN- PACHECO[1];
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Abstract: High glucose (HG) and hypoxia/reperfusion (HR) injury are the most common pathologies worldwide. The damage caused by HG and HR injury is linked to an exaggerated production of reactive oxygen species (ROS). In this respect, it is well known that the NADPH oxidases of the NOX family are the major sources of ROS in cardiomyocytes. Recent studies have shown that peroxisome proliferator activated receptors (PPARs) can be considered as potential therapeutic targets in processes that involve HR and HG. PPARs are members of the nuclear receptor superfamily, whose activation promotes the expression of genes encoded in specific DNA sequences. The PPAR-α is found predominantly in heart, where they participate in lipid metabolism acting as a sensor of the levels of free fatty acids. Each of the PPARs can be activated by specific ligands. Fenofibrate and other lipid-lowering agents are considered specific for activating the alpha subtype. Therefore, the objective of this work is to study whether the stimulation of alpha PPARs, by fenofibrate decreases the production of oxidative stress, increases expression and activity of the enzymes involved in the regulation of the redox state, and improves the ultrastructure of cardiomyocytes in a model of cardiomyocytes subjected to HR (2 hours hypoxia/1 hour reperfusión) and HG. For that purpose, isolated neonatal rat cardiomyocytes were divided into 2 main groups: (1) control and (2) fenofibrate (10µM). Both groups were subdivide into: (1) sham; (2) HG (25mM); (3) HR (with a coverslip); and (4) HG/HR. Our results indicate that cell viability decreases in the groups of cardiomyocytes subjected to HG, HR and both conditions while treatment with fenofibrate restores the viability to control levels. When evaluating the main source of ROS we observed that in HG o HR the cytosolic as well as the membrane subunits of the NADPH oxidase are increased while

fenofibrate treatment decreases them. The expression of the antioxidant enzymes SODCu^{2+}/Zn^{2+} and SODMn^{2+} and the antioxidant capacity are increased in the cadiomyocytes treated with the activator of PPAR-α, while in the HG, HR or both the expresion is diminished. Due to high oxidant stress, important molecules in the cardiomyocyte are damaged such as nitric oxide endothelial synthase (eNOS) and DNA; fenofibrate treatment protects both eNOS and DNA. We also observed a decrease in the production of MDA in the cardiomyocytes treated with the drug, which indicates a lower amount of damage to the membranes of the cardiomyocytes. These results, taken together suggest that fenofibrate treatment protects the cardiomyocyte from oxidative stress produced by HG, HR and the combination of both conditions.

Disclosures: F. Cortés López: None. **M.D. Ibarra- Lara:** None. **M.A. Sánchez- Mendoza:** None. **E. Soria-Castro:** None. **M. Sánchez- Aguilar:** None. **L. del Valle- Mondragón:** None. **L.G. Cervantes- Pérez:** None. **V. Ramirez- Gonzalez:** None. **V. Castrejón:** None. **A. Sánchez- López:** None. **G. Pastelín- Hernández:** None. **D. Centurión- Pacheco:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.09/F35

Topic: C.08. Ischemia

Support: SFI (13/IA/1881)

Title: Activation of AMP-activated protein kinase mediates ischaemic neuronal injury through modulation of miR-210

Authors: *S. L. PFEIFFER[1], P. WEISOVÁ[1], U. MAMRAK[2], S. HAUNSBERGER[1], A. RESLER[1], A. BEňOVÁ[3], B. D'ORSI[4], G. CHEN[1], H. DUSSMANN[1], B. HENNESSY[5], N. PLESNILA[2], J. H. PREHN[1];
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Abstract: Progressive neuronal injury of the ischaemic penumbra after stroke is associated with glutamate-induced depolarisation, energetic stress and activation of AMP-activated protein kinase (AMPK). AMPK activation in neurons in response to ischaemic and excitotoxic injury is a critical regulator in cellular energy function in response to stress, triggering both pro-survival and pro-apoptotic signalling. The duration and level of neuronal AMPK activity is a pivotal factor in the balance between neurotoxic and neuroprotective roles but the molecular switches that determine these roles, whether neurons undergo necrosis, apoptosis or tolerate an excitotoxic

insult, are not well understood. Here, we aimed to identify molecular signatures associated with AMPK activation in response to progressive neuronal injury regulating key downstream signalling pathways involved in ischaemia-reperfusion injury. We identified significantly increased levels of miR-210 in an *in vitro* model of acute AMPK activation in primary cortical neurons and subsequently observed a robust induction of miR-210 after ischaemic stroke in an *in vivo* model of transient middle cerebral artery occlusion in wild type mice, accompanied by a reduction in p70 ribosomal S6 kinase (p70S6K) expression. RPPA analysis of protein expression changes in brain tissue of wild type mice following intracerebroventricular (icv) administration of a locked nucleic acid (LNA) oligonucleotide miR-210 mimic revealed decreased expression of phosphatase and tensin homologue (PTEN), p70S6K and ribosomal protein S6 (rpS6). Utilising models of NMDA receptor overactivation in primary neurons, we detected rapid induction of miR-210 accompanied by suppression of p70S6K in response to AMPK activation. Furthermore, suppression of p70S6K activity was observed to exacerbate glutamate-induced apoptotic injury in primary neurons. Collectively, these results suggest a novel mechanism of miR-210 in the regulation of apoptosis and modulation of protein synthesis as a result of prolonged energetic stress and acute activation of AMPK, regulating key downstream signaling pathways representing a key modulator and novel regulatory target in ischaemic neuronal injury.

Disclosures: S.L. Pfeiffer: None. **P. Weisová:** None. **U. Mamrak:** None. **S. Haunsberger:** None. **A. Resler:** None. **A. Beňová:** None. **B. D'Orsi:** None. **G. Chen:** None. **H. Dussmann:** None. **B. Hennessy:** None. **N. Plesnila:** None. **J.H. Prehn:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.10/F36

Topic: C.08. Ischemia

Support: CONACYT 239516-CB

Title: Absence of Aryl hydrocarbon receptor promotes a effect neuroprotective in response to brain ischemia insult

Authors: *R. CASTANEDA ARELLANO[1], L. G. GARCIA-LARA[2], Q. D. ÁNGELES-LÓPEZ[3], F. PÉREZ-SEVERIANO[6], G. ELIZONDO-AZUELA[4], S. GONZÁLEZ-POZOS[5], J. V. SEGOVIA-VILA[3];
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Abstract: The aryl hydrocarbon receptor (Ahr) plays an important role in a wide variety of pathological phenomena such as ischemia, trauma and neurodegenerative diseases by modulating signalling pathways leading to apoptosis. Based on these data, we studied the role of Ahr in brain ischemia model. First, we induced a Bilateral Common Carotid Artery Oclussion (BCCAO) in male mice (*Wild-type* $Ahr^{+/+}$ *and Ahr-null* $Ahr^{-/-}$). In these experimental conditions, five days post-injury, we performed transmission electron microscopy to provide insights into hippocampus CA1 of $Ahr^{-/-}$ mice vs *Wild-type* littermates, before and after the ischemic injury. We found that synaptic loss and neuronal death is lower in $Ahr^{-/-}$ CA1 after damage. Aditionally, we found that abscence of Ahr mice induced a partial protection of Fluoro-Jade positive cells in comparison with $Ahr^{+/+}$, in hippocampus. Thus, showed a very intense GFAP staining from 24 hrs to 5 days post-injury in $Ahr^{+/+}$ group. Furthermore, *Ahr-null* mice ($Ahr^{-/-}$) showed a lower GFAP-expression. This neuroprotective effect observed against the brain ischemia provoked by BCCAO, was associated with increasing of Claudin and KatII expression. Together, these data suggest that the abscence of the Ahr could participate as a neuroprotective mechanism in ischemic injury.

Disclosures: R. Castaneda Arellano: None. **L.G. Garcia-Lara:** None. **Q.D. Ángeles-López:** None. **F. Pérez-Severiano:** None. **G. Elizondo-Azuela:** None. **S. González-Pozos:** None. **J.V. Segovia-Vila:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.11/F37

Topic: C.08. Ischemia

Support: Werner Otto Foundation
Schilling Foundation

Title: The prion protein is highly abundant in a cleaved form on cerebral extracellular vesicles and may regulate their neuronal binding. Potential implications in hypoxic stroke

Authors: S. BRENNA[1], H. C. ALTMEPPEN[2], B. MOHAMMADI[2], F. SCHLINK[1], P. LUDEWIG[1], C. SCHNEIDER[3], M. GLATZEL[2], *B. PUIG[1], T. MAGNUS[1];
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Abstract: Extracellular vesicles (EVs) are important tools in cell communication, participating in several physiological and pathological processes. In stroke, one of the leading causes of mortality and disability in the world, it has been observed that EVs are increased in the plasma of affected patients and that the presence of certain cargos correlates with tissue damage degree.

Thus, brain cells are very active in releasing EVs after stroke, but little is known about the composition of brain EVs and which cells are the main contributors to their release in steady-state conditions and after ischemia. The prion protein (PrP) a GPI-anchored protein highly enriched at synapses and in EVs, has been linked to a neuroprotective role after ischemic insults. PrP^{C} undergoes several physiological cleavages which are highly conserved through evolution, indicating important functions. In the present study, we have isolated EVs from wild-type C57BL/6 mice brains and from the affected hemispheres of mice subjected to transient middle cerebral artery occlusion (tMCAO) and shams, as a stroke model. We show that in steady-state conditions the brain cells with the highest EV release are microglia and oligodendrocytes. Interestingly, EVs are highly enriched in the C1 fragment of PrP. As the C1 fragment exposes a hydrophobic stretch at its N-terminus -as certain viral fusion proteins- we hypothesized a possible function in EV-to-cell tethering and uptake. We, therefore, incubated primary neurons with EVs isolated from either WT or $PrP^{0/0}$ mice at different time points. Immunofluorescence analysis showed that, contrary to our hypothesis, EVs form $PrP^{0/0}$ were most efficiently taken up, not only by neurons but also by microglia. Our experiments demonstrate that PrP has a function in regulating EV uptake by neurons and might be involved in the recognition of self by microglia, roles that may be particularly associated with the C1 fragment. Moreover, after 24h of stroke, microglia release twice more EVs than in steady-state conditions and there is also an increased contribution by oligodendrocytes and astrocytes. On the contrary, neurons have a tendency to decrease their EVs release, probably because they are most affected by the ischemic insult. The PrP-to-C1 pattern is not significantly altered upon stroke. To our knowledge, this is the first study to demonstrate that brain-derived EVs are highly enriched in PrP-C1 with potential implications in EV uptake. We also show that microglia and oligodendrocytes are the main sources of brain EVs in steady-state conditions, and after 24h of stroke microglia, oligodendrocytes, and astrocytes EVs are importantly increased.

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Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.12/F38

Topic: C.08. Ischemia

Title: Effect of myocardial infarction induced PS2 overexpression in rat brain and the abnormal events associated with calcium imbalance

Authors: *J. K. SWAMINATHAN, A. KAMALABAI, A. MUTHUSWAMY;
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Abstract: Previous reports had proved that MI, induced the overexpression of PS2 by dysregulating calcium balance invivo. PS2 overexpression is believed to play an important role in Ca^{2+} imbalance at various abnormal conditions that form the major reason behind various pathophysiological conditions. MI induced arrhythmia and myocardial stunning is proved to be the resultant of Ca^{2+}overload in heart. As a resultant, brain that demand more oxygen may be the most susceptible organ that may be affected due to MI. Further it is reported that PS2 overexpression is capable of producing Ca^{2+} leak in the sarcoplasmic reticulum and mitochondria that are classical hallmark of neurodegenerative diseases like Alzheimer's disease. Hence current study propose the link between PS2 over expression, Ca^{2+} leak and abnormal intracellular signaling events in brain as a consequential event of myocardial infarction. Therefore, activation and interaction of various signaling pathways during this event was analyzed using tools like STRING and CYTOSCAPE for the protein-protein interaction network that was constructed based on conditions of PS2 over expression. LAD ligation was performed to imitate experimental Myocardial Infarction and the target proteins derived from insilico experiments were investigated in rat brain to reveal inflammatory response. It was observed that there was significant alterations in inflammatory markers viz., inerleukins, NFκB etc.,. More interestingly, the PS2 overexpression in rat heart during MI has created imbalance in brain calcium. Further, the apoptotic markers were significantly elevated indicating abnormalities in brain of MI induced rat when compared to control animals. These results thereby conclude the influence of PS2 in calcium overload and imbalance in rat brain thereby implicating susceptibility for neurodegeneration and cognitive abnormalities.

Disclosures: J.K. Swaminathan: None. **A. Kamalabai:** None. **A. Muthuswamy:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.13/F39

Topic: C.08. Ischemia

Title: Examining the mitochondrial network within neurons following cardiac arrest and resuscitation

Authors: *K. J. MAHERAS[1], T. H. SANDERSON[2];
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Abstract: The primary cause of death in post-cardiac arrest patients is brain injury. While the return of spontaneous circulation (ROSC) is essential to preserve the ischemic tissue, it also, paradoxically, exacerbates brain injury and heightens mortality rates. The pathophysiology of post-cardiac arrest injury involves a complex cascade of molecular events revolving around mitochondria. Mitochondrial dynamics play a causal role amplifying injury through disruption of calcium homeostasis, reactive oxygen species (ROS) generation, and/or activation of cell death pathways. Mitochondrial quality control depends on the delicate balance of fission, fusion, and targeted autophagy of unthrifty mitochondrial (mitophagy). This purposeful equilibrium is known to play a pivotal role in maintenance of healthy neurons, however, controversy exists as to the role of mitochondrial quality control under duress, namely in the progression of post-ischemic brain injury.
A novel binary-based fluorescence assay was developed in which the expression of functionally inert, tandem mCherry-GFP is fused to the targeting sequence of the mitochondrial outer membrane protein, Fis1. Under stable conditions, both red and green fluorophores are expressed. Upon mitophagy when mitochondria fuse with lysosomes for degradation, the mCherry fluorescence remains stable, but GFP becomes quenched in the acidic environment. Thus, a fluorescence ratio can be utilized to quantify mitochondrial quality control flux in physiologic and pathologic contexts. A transgenic mouse model, Mito-QC, was generated, to investigate the mitochondrial network and mitophagy *in vivo*. Herein, we have combined the Mito-QC mouse with our novel mouse model of cardiac arrest/resuscitation to examine mitophagic flux within neurons of the CA1 hippocampus at various time points post ROSC. This refined exploration sheds light into the oscillating network of mitochondria under stress and may provide novel insight for therapeutic interventions to reduce neuronal injury in post-ischemic brain.

Disclosures: K.J. Maheras: None. **T.H. Sanderson:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.14/F40

Topic: C.08. Ischemia

Support: Heart and Stroke Foundation of Canada

Title: Investigation of Na+/K+-ATPase isoforms in higher and lower brain regions following focal ischemia in mice

Authors: *C. A. LOWRY[1], B. M. BENNETT[2], R. D. ANDREW[2];
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Abstract: Clinically and experimentally, higher gray matter is more susceptible to acute ischemic injury than lower gray matter. Previous work in our laboratory in which rodent brain slices were exposed to ischemia has supported this phenomenon. Fifteen minutes of simulated ischemia (oxygen-glucose deprivation) causes spreading depolarization (SD) which irreparably damages higher brain neurons (such as neocortical and hippocampal pyramidal cells). In contrast, neurons of the hypothalamus and brainstem resist SD-induced damage. Discovering mechanisms which contribute to the brainstem's resilience may inform targets for improved survival of higher brain neurons. As failure of the Na+/K+-ATPase is a key event following ischemia, we hypothesized that differential regional susceptibility of the brain to ischemia might be explained in part by variable expression of Na+/K+-ATPase isoforms, which differ in pumping efficiency under low energy conditions. Our objective was to examine Na+/K+-ATPase alpha1 and alpha3 mRNA and protein levels in various higher and lower brain regions in both naïve mice and those that have undergone a focal stroke (middle cerebral artery occlusion - MCAo). Analysis of mRNA has shown that under basal conditions, expression of the ischemia-vulnerable alpha1 isoform in neocortex is on average 2.2x higher than the alpha3 isoform ($n=10$, $p<0.05$), whereas in the brainstem, the ischemia-resistant alpha3 isoform is on average 2x higher than the alpha1 isoform ($n=10$, $p<0.05$). Parallel protein expression analyses are consistent with these findings, yielding a significantly lower alpha1/alpha3 ratio in the brainstem compared to the neocortex ($n=10$, $p<0.05$), indicating proportionally more alpha3 in this region. Preliminary data from mice undergoing a 30-minute MCAo shows that 24-hours post-stroke, mRNA expression of the alpha1 isoform decreases significantly in the ipsilateral compared to contralateral hemisphere. We are currently analyzing alpha1 and alpha3 mRNA and protein levels in various higher and lower brain regions of mice undergoing MCAo, at various time points after occlusion (6-, 12-, and 24-hours). We hypothesize that alpha1 and alpha3 isoform mRNA and protein levels will decrease and increase, respectively, following stroke, particularly in the neocortex and striatum, whereas expression in the brainstem will remain unchanged. Understanding how Na+/K+-ATPase isoforms differ in their expression in response to metabolic stress will yield insights into how such differences protect neurons during ischemia.

Disclosures: C.A. Lowry: None. **B.M. Bennett:** None. **R.D. Andrew:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.15/F41

Topic: C.08. Ischemia

Title: Multiplex profiling of secreted factors in the cerebrospinal fluid of Moyamoya disease patients

Authors: *K. ABHINAV[1], A. LEE[2], A. PENDHARKAR[1], Y. ROSENBERG-HASSON[1], H. UCHINO[1], M. Y. CHENG[3], G. K. STEINBERG[4];
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Abstract: Background: Moyamoya disease (MMD) is a rare, chronic cerebrovascular disease characterized by progressive occlusion of the internal cerebral arteries. The exact underlying pathogenesis of MMD remains unclear. We comprehensively profiled 62 secreted factors in the cerebrospinal fluid (CSF) of MMD patients, including angiogenic growth factors, cytokines and chemokines. Our objective was to identify key secreted factors associated with MMD including in relation to subtypes and correlate the levels with the extent of neovascularization and functional outcomes following a revascularization procedure.
Methods: CSF from angiographically confirmed MMD was collected. All MMD patients had undergone revascularization procedure usually in the form of direct superficial temporal artery to middle cerebral artery bypass. Three groups were classified: 32 controls (Chiari or microvascular decompression), 37 patients with MMD (ischemic) and 34 patients with MMD (hemorrhagic). CSF proteins were measured using the multiplex luminex assay. Differential differences were conducted with least mean square with CHEX4 as a covariate. All p-value are corrected by Tukey test. An ELISA was also used to quantitate the level of cellular retinoic acid binding proteins (CRBP1) in CSF samples. Functional outcomes were assessed using the modified Rankin scale score (mRS) and Matsushima criteria was used for grading the neovascularization.
Results: 40 of 62 secreted factors were significantly elevated in both MMD groups in relation to controls. We identified secreted factors not reported previously. The common top highly secreted CSF proteins include platelet-derived growth factor bb, chemokine ligand 5 and plasminogen activator inhibitor 1 ($p<0.001$). CRBP1 and other growth factors such as brain-derived nerve growth factor, basic fibroblast growth factor and vascular endothelial growth factor were elevated in both MMD groups ($p<0.01$). These factors are involved in the inflammatory responses including activation and migration of immune cells and angiogenesis. We also observed several unique genes that differed between the two MMD subtypes (ischemic versus hemorrhagic).
Conclusions: CSF analysis revealed that a number of cytokines, chemokines and growth factors are elevated in the MMD. Increased CRBP1 in MMD is consistent with the literature and potentially causes the increase of growth factors and cytokines contributing to the pathogenesis. Further analysis will elucidate the relationship between these factors and the grade of angiogenesis and functional outcomes.

Disclosures: K. Abhinav: None. **A. Lee:** None. **A. Pendharkar:** None. **Y. Rosenberg-Hasson:** None. **H. uchino:** None. **M.Y. Cheng:** None. **G.K. Steinberg:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: C.08. Ischemia

Support: DGAPA-PAPIIT IN226617
CONACYT A1-S-13219

Title: Dna methylation inhibition promotes axonal growth and neuronal survival in a murine cerebral ischemia model

Authors: *I. PONCE, L. VALENCIA-LÓPEZ, R. SANTANA-MARTÍNEZ, L. TOVAR-Y-ROMO;
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Abstract: DNA methylation is a critical regulator of neuronal function through transcriptional regulation. Even though this mechanism has a central role in neurovascular-related disorders like ischemic stroke, few studies have addressed at the molecular resolution the overall genetic and epigenetic changes of these complex phenomena, and in events like reperfusion damage that occurs after ischemic stroke, these processes are practically unknown. In an experimental model of cerebral ischemia produced by the transient occlusion of the middle cerebral artery (MCAO) in CD-1 mice, we evaluated the expression of several genes involved in the regulation of neuronal death and recovery. We also studied how these genes changed their expression profile when DNA methylation was ablated by the inhibition of DNA methyltransferases induced by the i.c.v of 30 µg 5-azacytidine. We determined the effects of inhibiting DNA methyltransferases on behavioral tests to assess the neurological status of the animals in terms of balance, coordination, grip strength and skilled motor function following stroke. Also, we characterized the changes in expression of several signaling pathways implicated in the regulation of reactive oxygen species balance, axonal growth and neuronal survival analyzed by RT-qPCR. We correlated all these changes to promoter methylation analyzed by methylation-specific PCR at 24 h, 72 h, and 7 d after MCAO. These results contribute to elucidate the overall changes induced by ischemia and reperfusion in terms of transcription and DNA methylation in cerebrovascular accidents and will add to the understanding of how reperfusion damage caused after the stroke is resolved.

Disclosures: I. Ponce: None. **L. Valencia-López:** None. **R. Santana-Martínez:** None. **L. Tovar-y-Romo:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.17/F43

Topic: C.08. Ischemia

Support: CalciGenix

Title: Neuroprotective effects of apoaequorin on ischemic stroke

Authors: *C. W. SMIES[1], J. R. MOYER, Jr[1,2];
[1]Psychology, [2]Biol. Sci., Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Strokes are commonly experienced by aged individuals, and due to the growth of this subpopulation there is an increased need to investigate the cellular and molecular mechanisms associated with stroke. Ischemic strokes are the result of an occlusion that prevents tissue from receiving oxygen and glucose, which can lead to excitotoxicity through calcium dysregulation (Choi, 1999). Overexpression of the calcium binding protein calbindin D28K has been shown to reduce calcium-induced excitotoxicity caused by cerebral ischemia (Yenari et al., 2001). Given that ischemic insults result in calcium mediated excitotoxicity and since proteins that sequester calcium (Ca^{2+}) can reduce ischemic damage, we investigated the effects of the calcium binding protein apoaequorin (AQ) as a possible neurotherapeutic in an *in vitro* stroke model. Dorsal hippocampal infusion of AQ 1d prior to *in vitro* ischemia reduces cell death and increases cytokine mRNA expression (e.g. tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10; Detert et al., 2013)). TNF-α is a cytokine that is involved in ischemic preconditioning (Wang et al., 2000). Ischemic preconditioning is phenomena that results in reduced damage from an ischemic insult as a consequence of a preceding smaller ischemic insult. Therefore, we further investigated the role of these cytokines as possible candidates involved in AQ's neuroprotective effect. To explore the link between AQ's neuroprotective effect and cytokine expression in the rodent brain the current study aims to determine when cytokine expression occurs and how this is related to reduced cell death as a result of a single infusion of AQ. A combination of RT-qPCR, Western blotting, and *in vitro* ischemia are used to investigate cytokine mRNA, cytokine protein, and cell death respectively. Animals are bilaterally cannulated targeting the dorsal hippocampus and infused with either Ca^{2+}-free artificial cerebral spinal fluid (aCSF) or AQ and sacrificed 1h, 12h, 1d, or 2d post-infusion. The link between reduced cell death and timing of cytokine expression may reveal possible points of intervention for those who are at risk for suffering from ischemic strokes.

Disclosures: C.W. Smies: None. **J.R. Moyer:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.18/F44

Topic: C.08. Ischemia

Support: NIH RO1 NS101960
NIH RO1 NS099531
NIH RO1 NS109459

Title: Deletion of long noncoding RNA FosDT ameliorates post-ischemic functional deficit in both sexes independent of age

Authors: *S. L. MEHTA[1], T. KIM[2], K. MORRIS-BLANCO[3], S. BATHULA[1], A. K. CHOKKALLA[3], R. VEMUGANTI[1];
[1]Neurolog. Surgery, Univ. of Wisconsin, Madison, WI; [2]Neurolog. Surgery, Univ. of Wisconsin Madison, Madison, WI; [3]Neurolog. Surgery, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Transient focal ischemia induces extensive spatiotemporal changes in expression levels of protein-coding and many classes of noncoding RNAs including long non-coding RNAs (lncRNAs). The significance of lncRNAs expression to the neurologic outcome after stroke although is not clear, we recently observed that lncRNA called Fos downstream transcript (FosDT, transcribed from Fos locus) is significantly induced in the brain following ischemic stroke. We further showed that FosDT scaffolds the transcription factor REST and its corepressors enabling REST-mediated suppression of neural genes and thereby providing neuroprotection and better functional outcome following stroke. To understand its roles, we further investigated whether 1) FosDT expression is developmentally and stroke regulated, 2) whether rodent knockouts of FosDT are viable, fertile and developmentally normal and 3) whether post-ischemic targeting of FosDT is a viable strategy to curtail stroke brain damage. We observed that FosDT is abundantly expressed in both peripheral organs and brain with cerebral cortex showing the highest levels. FosDT expression is also developmentally regulated with adults showing higher expression than neonates. Its expression is highest in the cerebral cortex of adult as compared to the P7 stage. We also found that transient focal ischemia-induced the expression of FosDT independent to age and sex in young adult and aged male and female animals. When FosDT was knocked-out in rats using CRISPR/Cas9, there were no developmental effects. FosDT knockout rats showed normal body weight and fertility compared to wildtype rats. However, when challenged with a focal cerebral ischemic insult, FosDT knockouts showed better survival and smaller infarcts than wild-type controls at 7 days of reperfusion. FosDT deletion also significantly reduced post-ischemic functional deficits and improved sensory-motor recovery. Similarly, post-ischemic silencing of FosDT also significantly

improved the functional deficits and sensory-motor recovery in both sexes independent of age. Thus, our results show that FosDT induction could modulate the post-stroke functional outcome and targeting lncRNAs such as FosDT is a viable strategy to minimize post-stroke brain damage.

Disclosures: S.L. Mehta: None. **T. Kim:** None. **K. Morris-Blanco:** None. **S. Bathula:** None. **A.K. Chokkalla:** None. **R. Vemuganti:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.19/F45

Topic: C.08. Ischemia

Support: NIH RO1 NS1231513

Title: Impact of age and sex on alpha-synuclein inhibition induced post-stroke recovery

Authors: *B. CHELLUBOINA, T. KIM, J. KIM, S. BATHULA, R. VEMUGANTI; Neurolog. Surgery, Univ. of Wisconsin, Madison, WI

Abstract: We recently investigated the pathological role of α-synuclein (α-syn) in post-stroke secondary brain damage. Our studies identified that α-syn suppression significantly mitigated the post-ischemic oxidative stress, apoptosis and mitochondrial dysfunction in young, male rodents. Based on the critical role of α-syn in ischemic brain damage, we extended our studies to evaluate if α-syn inhibition can protect post-stroke brain in both sexes. Mice were subjected to transient middle cerebral artery occlusion (MCAO) and then administered with a α-syn-specific siRNA cocktail via retro-orbital route at 30 min after reperfusion. α-syn mRNA and protein levels were analyzed by real-time PCR and Western blots. Post-stroke motor deficits were evaluated with rotarod and beam walk tests at 1 to 7 days of reperfusion, and infarct volume was measured with cresyl violet-stained brain sections at 7 days of reperfusion. Cellular changes after ischemia were examined using immunofluorescence staining. Following transient MCAO, α-syn levels were significantly up-regulated in both young and aged mice at 24hr of reperfusion. Silencing α-syn by α-syn siRNA after reperfusion significantly decreased the infarction, improved the motor function and led to higher rate of survival in young mice of both sexes compared to control siRNA-treated mice. Thus, our studies show that α-syn plays a critical role in preventing neuronal death following stroke irrespective of sex. Furthermore, α-syn-specific siRNA inhibition protects the brain after stroke in both sexes. Preventing α-syn expression is a potential therapeutic target to minimize post-stroke brain damage.

Disclosures: B. Chelluboina: None. **T. Kim:** None. **J. Kim:** None. **S. Bathula:** None. **R. Vemuganti:** None.

Poster

049. Ischemic Stroke I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 049.20/F46

Topic: C.08. Ischemia

Support: EU European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant agreement No.KK.01.1.1.01.0007, CoRE – Neuro
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Multimodal imaging was done at Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb School of Medicine.

Title: Longitudinal multimodal *in vivo* imaging reveals the dynamic of the ischemic injury in toll-like receptor 2 (Tlr2)-deficient mouse

Authors: *S. GAJOVIC[1], M. DOBRIVOJEVIC RADMILOVIC[1], D. GORUP[1], S. SKOKIC[1], A. GLASNOVIC[1], P. JOSIC[1], J. KRIZ[2];
[1]Croatian Inst. for Brain Res., Univ. of Zagreb Sch. of Med., Zagreb, Croatia; [2]Laval Univ., Quebec, QC, Canada

Abstract: Inflammatory response after ischemic brain injury is mediated by Toll-like receptors (TLR) located on the microglia. Different studies of Tlr2-deficient mice showed different, even opposing results, suggesting a dual, time-dependent effect. Therefore, the aim of the current study was to clarify this issue by performing a longitudinal *in vivo* multimodal magnetic resonance imaging and bioluminescence imaging of the ischemic injury in Tlr2-deficient mice. The consequences of the transient middle cerebral artery occlusion (MCAO) were compared between Tlr2-deficient and C57Bl6 (WT) mice. The animals were imaged at multiple time points by a 7T BioSpec 70/20 USR MRI system and with optical imager Perkin Elmer IVIS Spectrum. Gap43-luc/gfp bioluminescence was imaged to measure neurorepair and caged luciferase VivoGlo (Promega) was imaged to reveal caspase 3 and 7 activity and measure apoptosis. The functional consequences were assessed by behavioral testing and neurological scoring. At day 28 after the ischemia the animals were sacrificed, and the brains were processed for histological analysis. The survival analysis showed lower mortality for Tlr2-deficient group compared to WT group. Most of the non-surviving animals were lost during the first week after surgery. The WT non-survivors had significantly higher neurological score than the animals which reached day 28

after surgery, which was not the case for Tlr2-/- mice. The Tlr2-/- group showed higher neurological deficit in the acute phase, bigger lesion volume in the first week, and higher edema index in the first 3 days reaching similar levels as WT at day 7 after surgery. In Tlr2-deficient mice Gap43 bioluminescence was higher until 28 days after ischemia, and caspase 3 and 7 activity increased 14 days onward as compared to the wild type animals. In conclusion, longitudinal multimodal imaging revealed that modulated neuroinflammation due to Tlr2 deficiency increases the ischemic lesion and apoptosis, but, interestingly, at the same time enhances the survival rate and Gap43-dependent repair processes.

Disclosures: S. Gajovic: None. **M. Dobrivojevic Radmilovic:** None. **D. Gorup:** None. **S. Skokic:** None. **A. Glasnovic:** None. **P. Josic:** None. **J. Kriz:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.01/G1

Topic: C.10. Brain Injury and Trauma

Support: NIH-R21-NS096515
Arizona Alzheimer's Consortium
T32-AG044402

Title: Traumatic brain injury and Alzheimer's disease in aged mice leads to similar increases in sleep and peripheral Cd115 expression

Authors: *M. SABER[1,2], Y. HUR[1,2], O. N. KOKIKO-COHRAN[3], R. K. ROWE[1,2,4], J. LIFSHITZ[1,2,4];
[1]Child Hlth., Univ. of Arizona Col. of Med. - Phoenix, Phoenix, AZ; [2]Barrow Neurolog. Inst. at Phoenix Children's Hospital, Phoenix, Phoenix, AZ; [3]The Ohio State Univ. Neurolog. Inst., Columbus, OH; [4]Phoenix Veterans Affairs Hlth. Care Syst., Phoenix, AZ

Abstract: The bidirectional feedback between peripheral and central inflammation drive pathological signaling in traumatic brain injury (TBI) and Alzheimer's disease (AD) with direct effects on sleep. Identifying specific inflammatory markers that link TBI, AD, and changes in sleep may elucidate mechanisms of chronic pathophysiology. Practical interventions to reduce inflammation may improve sleep and outcomes. We hypothesized that TBI in aged wild-type mice leads to inflammation and disrupted sleep similar to an AD model and reduction of peripheral inflammation through remote ischemic conditioning (RIC; transient restriction of blood flow to a limb) would resolve sleep disruption.
18-month 3xTg-AD (B6;129-Psen1 Tg[APPSwe,tauP301L]1Lfa; n= 22) and wild type (n= 34) mice were acclimated to non-invasive sleep cages. Wild-type mice received midline fluid

percussion injuries (mFPI; n=27) or were naive controls (n=7). One hour post-injury mice were randomly assigned to a treatment cohort (3xTg-AD RIC, 3xTg-AD no-RIC, TBI RIC, TBI no-RIC). Mice received 4x5 minute sessions of RIC or control anesthesia. Blood was taken 1day post-injury (DPI) and mice were returned to sleep cages. At 5 DPI cognitive and affective behaviors were tested prior to tissue collection.
TBI and 3xTg-AD mice had significantly more cumulative sleep during the dark cycle compared to naive controls ($F(2,43)=9.62$, $p=0.0004$) with no effect of RIC at 1 DPI. At 5DPI, significantly more Cd115+ monocytes were in spleens and blood of wild-type TBI and 3xTg-AD mice compared to naive controls (spleen: $F(2,23)=4.742$, $p=0.0189$; blood: $F(2,23)=3.617$, $p=0.0424$), but were not significantly different between the two models. Further analysis will determine whether peripheral CD115+ expression predicts increases in sleep and whether reduction of Cd115+ ameliorates sleep changes. These data provide a new mechanistic link between TBI and AD pathophysiology.

Disclosures: M. Saber: None. **Y. Hur:** None. **O.N. Kokiko-Cohran:** None. **R.K. Rowe:** None. **J. Lifshitz:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.02/G2

Topic: C.10. Brain Injury and Trauma

Title: Somatostatin interneuron dysfunction in the orbitofrontal cortex is associated with cognitive inflexibility after traumatic brain injury

Authors: *A. NOLAN[1], V. S. SOHAL[3], S. ROSI[2];
[2]Brain and Spinal Injury Ctr., [1]Univ. of California San Francisco, San Francisco, CA; [3]Dept. of Psychiatry, U. California, San Francisco, San Francisco, CA

Abstract: Traumatic brain injury (TBI) is a leading cause of neurologic disability and the most common associated cognitive deficits affect prefrontal cortex (PFC)-dependent functions such as: attention, working memory, social behavior, and mental flexibility. Despite this prevalence, little is known about the pathophysiology that develops in frontal cortical microcircuits after TBI. In our mouse model of frontal lobe contusion that recapitulates aberrant mental flexibility as measured by deficits in rule reversal learning, we investigated if selective intrinsic deficits in neuronal firing are associated with cognitive inflexibility after TBI. Patch clamp recordings were performed in layer V pyramidal and inhibitory neurons two months after injury in the orbitofrontal cortex (OFC) of male mice. The principal output neurons, the pyramidal neurons, showed minimal changes in function with only a minor reduction in the action potential threshold in TBI compared to sham cohorts; and the fast-spiking parvalbumin-expressing

interneurons had identical action potential (AP) and passive membrane properties in both groups. In contrast, the non-fast spiking somatostatin-expressing (SOM+) inhibitory neurons exhibited a striking vulnerability to injury with a wider AP half width, reduced falling AP slope, and an increase in the adaptation index leading to reduced excitability and fewer action potentials produced at depolarizing current steps after TBI. Given that SOM+ inhibitory neurons play a direct role in feedback inhibition and dampening of excessive activity in cortical circuits, dissecting why these inhibitory neurons are selectively vulnerable to injury may be a key component in restoring cognitive function after TBI.

Disclosures: A. Nolan: None. **S. Rosi:** None. **V.S. Sohal:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.03/G3

Topic: C.10. Brain Injury and Trauma

Support: NIH NINDS R01NS096143

Title: Evaluating cathepsin B and its role in neuronal membrane disruption following diffuse brain injury in rats

Authors: *M. L. HERNANDEZ[1], M. MARONE[2], K. M. GORSE[1], A. D. LAFRENAYE[1]; [1]Anat. and Neurobio., [2]Pharmacol. and Toxicology, VCU, Richmond, VA

Abstract: Traumatic brain injury (TBI) has consequences that last for years following injury and are associated with diffuse pathology. While TBI can precipitate a variety of diffuse pathologies, the mechanisms involved in injury-induced neuronal membrane disruption remain elusive. The lysosomal cysteine protease, Cathepsin B (Cath B), and specifically its redistribution into the cytosol upon membrane disruption has been implicated in cell death. Little is known about Cath B or neuronal membrane disruption chronically following diffuse TBI. Therefore, the current study evaluated Cath B and diffuse neuronal membrane disruption over a more chronic post-injury window. We evaluated Cath B in adult male Sprague-Dawley rats at 6h, 1d, 3d, 1w, 2w, and 4w following central fluid percussion injury (CFPI). As diffuse neuronal membrane disruption was consistent in layers V and VI of the lateral neocortex following CFPI, all assessments were done in this region. Expression of Cath B, as well as Cath B-associated pro (Bak and AIF) and anti-apoptotic (Bcl-xl) proteins, were assessed using western blot analysis. Cath B activity was assessed with a fluorescent substrate, z-F-R-AMC, in sham and CFPI animals from 6h-4w. Subcellular localization of Cath B was also evaluated in the membrane disrupted population 6h-4w following CFPI using immunohistochemistry paired with quantitative image analysis. There was no difference in expression or activity of Cath B or any of

the associated proteins between sham and CFPI at any time post-injury. Immunohistological studies, however, showed a sub-cellular re-localization of Cath B at 2w and 4w post-injury in the membrane disrupted neuronal population. In order to focus on Cath B and its role in membrane disruption, we administered CA-074Me (10 μg/μL), a Cath B inhibitor, into the left lateral ventricle and assessed both left and right cortices, alongside the liver to evaluate systemic Cath B inhibition. There was a 45% unilateral inhibition in the left cortex in naïve rats 6h after infusion, but no inhibition in the right cortex or liver compared to vehicle control. These findings highlight aspects of the diffuse nature of CFPI and also denotes Cath B redistribution as a potential mechanism leading to membrane disruption chronically following TBI. Future studies using more direct targeting of Cath B in this diffuse injury with CA-074Me are warranted. Furthering our understanding of Cath B and its role in neuronal membrane disruption has implications for future treatment for TBI.

Disclosures: M.L. Hernandez: None. **M. Marone:** None. **K.M. Gorse:** None. **A.D. Lafrenaye:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.04/DP03/G4

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: C.10. Brain Injury and Trauma

Support: R01NS093073

Title: Axonal varicosity dynamics in central neuron mechanosensation and injury

Authors: *C. GU[1], C. SUN[2], J. P. RICE[3], Y. GU[4];
[1]Ohio State Univ., Columbus, OH; [3]Biol. Chemsitry and Pharmacol., [2]OSU, Columbus, OH; [4]Biol. Chemsitry and Pharmacol., The Ohio State Univ., Columbus, OH

Abstract: Mechanosensation underlying the senses of touch, hearing and balance, and pain, has been extensively studied, but little is known about how central neurons convert mechanical stimuli into changes in morphology and function. Our recent studies link axonal varicosity dynamics to central neuron mechanosensation. Axonal varicosities (swelling or beading) are enlarged, heterogeneous structures along axonal shafts. They are a key pathological feature believed to represent slow accumulation of axonal damage that occurs during irreversible degeneration, for example in mild traumatic brain injury (mTBI), Alzheimer's and Parkinson's diseases, and multiple sclerosis. Varicosities are also known to form at low levels under normal (i.e. nonstressed) brains, representing synaptic boutons. Although axonal varicosities can

profoundly impact action potential propagation to alter neuron-neuron communication, the mechanisms governing their initiation and development were poorly understood. We recently made a discovery about mechanical stress-induced axonal varicosities in central neurons. Using a novel biomechanical assay, we found that mechanical stress causes varicosity formation in the axons but not the dendrites of hippocampal neurons. Consistent with this finding, axonal varicosities can be induced in vivo in mouse brain by repetitive closed-skull traumatic brain impact in an mTBI model. We discovered that the formation of axonal varicosities by mechanical stress is unexpectedly rapid (within 5 sec) and reversible (about 20 min for half recovery). In this process, a mechanosensitive ion channel and a microtubule binding protein play key roles. Furthermore, our recent studies provide new insights into the regulation of the reversible-irreversible transition of axonal varicosities that are induced by mechanical stress. In sum, our findings in this research direction may not only illustrate a new form of neural plasticity, but also contribute to new strategies for treating mTBI and neurological diseases through enhancing the recovery of axonal varicosities.

Disclosures: **C. Gu:** None. **C. Sun:** None. **J.P. Rice:** None. **Y. Gu:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.05/G5

Topic: C.10. Brain Injury and Trauma

Support: CRC 1149

Title: Traumatic brain injury induces synaptic alterations dependent on the synaptic and ASD-related protein Shank3

Authors: *C. C. URRUTIA-RUIZ, T. M. BOECKERS;
Inst. for Anat. and Cell Biol., Ulm Univ., Ulm, Germany

Abstract: Traumatic brain injury (TBI) might lead to psychiatric disturbances, presumably associated with neuronal loss and/or an altered reconstitution of the synaptic connections, possibly leading to an imbalance between synaptic inhibition-excitation. At excitatory synapses, Shanks are large scaffolding proteins present at postsynaptic density (PSD) and are considered to be the “master organizer” of the PSD. Significant neurological and psychiatric conditions, such as Autistic Spectrum Disorders (ASD), have been attributed to mutations or loss of a copy of the Shank3 gene. This study is aimed to analyze synaptic and behavioral changes in response to TBI and in the context Shank3 loss. Mild TBI was performed in WT and Shank3Δ11(-/-) male mice at 8-10 weeks of age. After 5, 10, and 18 days the dendritic spines, excitatory synapses, and neuronal loss were analyzed. The expression of the stress-related hormone Corticotropin-

Releasing-Hormone (CRH) was also assessed. Finally, a behavioral analysis was performed to analyze motor, cognitive and autistic-like features. In WT animals mTBI induced specifically in the hippocampus no neuronal loss but less excitatory synapses and dendritic spines in the CA1 and CA3 regions. Interestingly, Shank3Δ11(-/-) mice, despite presenting a lower basal level of dendritic spines and excitatory synapses, exhibited no significant loss of synapses or dendritic spines after mTBI. Hippocampal CRH expression was highly upregulated in TBI-WT but not in TBI-Shank3Δ11(-/-) animals, indicating that stress response induced by mTBI is altered in these mice. In the open field arena, TBI-WT mice had anxiolytic or disoriented behavior, while TBI-Shank3Δ11(-/-) mice were unaffected. In the trace-fear conditioning paradigm, only WT mice displayed an enhanced fear learning. Whereas Shank3Δ11(-/-) mice exhibited an overall enhanced fear learning and enhanced contextual memory independent of mTBI. Neither mTBI function as a second hit for ASD in Shank3Δ11(-/-) animals nor induced autistic-like features in WT animals (assessed by self-grooming and Three-chamber test).Therefore, re-arrangements of the synaptic morphology and behavioral changes after TBI are influenced by the presence of Shank3, since TBI in the absence Shank3 induces an entirely different response at the synaptic and behavioral levels by no worsening of the original phenotype in a knock out-Shank3 model.

Disclosures: C.C. Urrutia-Ruiz: None. **T.M. Boeckers:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.06/G6

Topic: C.10. Brain Injury and Trauma

Support: NASU Biotechnology Grant

Title: Exclusive targeting of extrasynaptic NMDA receptors improves mice cognitive function after mild traumatic brain injury

Authors: A. CHAIKA[1], T. PIVNEVA[1,2], A. SAVTCHENKO[3], E. MOLOKANOVA[4], ***N. VOITENKO**[1,2];
[1]Sensory Signalling, Bogomoletz Inst. of Physiol., Kyiv, Ukraine; [2]Kyiv Academic Univ., Kyiv, Ukraine; [3]Nanotools Biosci., San Diego, CA; [4]NeurANO Biosci., SanDiego, CA

Abstract: Traumatic brain injury (TBI) results from severe or mild damage to the brain tissue from accidents or assaults. TBI often leads to impaired motor functions, amnesia, and cognitive dysfunctions, which dramatically reduces the quality of life, and societal productivity of TBI patients. As a result of TBI, neurons in the original damage area “spill” glutamate into the extracellular space, which initiates the vicious circle of events that is further amplified when the increasing number of neurons succumb to glutamatergic excitoxicity due to tonic overactivation

of glutamatergic NMDA receptors (NMDARs) outside the synaptic cleft. Therefore, the most neuronal damage in TBI happens hours and days after the injury. It also means that the secondary neuronal damage triggered by glutamate spill could be limited, prevented, and even reversed. NMDAR antagonists can protect neurons from glutamatergic excitoxicity, but with one caveat. To be effective and safe, NMDAR antagonists must only block pathophysiological process (extrasynaptic glutamatergic excitotoxity) and spare physiological process (synaptic transmission). With this goal in mind, we developed an exclusive antagonist of extrasynaptic NMDARs (eNMDARs) that due to its extended dimensions cannot enter the synaptic cleft and reach synaptic NMDARs, but can block eNMDARs (Savchenko et al., 2016). In our studies, we induced mild TBI in 3-month old mice according to a classical weight-drop model. We stereotaxically injected 2 μl of 20 nM AuM or the same amount of saline into hippocampus 24 hours after TBI. Four groups of experimental animals were used (control, TBI, TBI + saline, TBI + AuM). Following the 24-hour food deprivation, mice were subjected to T-maze tests at 2, 5 and 10 days after TBI. Our data revealed that the TBI animals treated with AuM performed significantly better that saline-injected TBI animals. Critically, by the day 5, AuM-treated TBI animals performed at least as well as control animals without TBI. Our findings highlight the critical importance of eNMDARs in TBI consequences and offer the opportunity to develop novel therapeutics to ameliorate the impact of TBI.

Disclosures: **A. Chaika:** None. **T. Pivneva:** None. **A. Savtchenko:** None. **E. Molokanova:** None. **N. Voitenko:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.07/G7

Topic: C.10. Brain Injury and Trauma

Support: NIH R01NS069861
NIH R01NS097750

Title: TLR4 regulation of excitation/inhibition balance in the uninjured and injured brain

Authors: ***S. NGUYEN**[1], D. SUBRAMANIAN[1], Y. LI[2], V. SANTHAKUMAR[1,2];
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[2]Pharmacology, Physiology, and Neurosci., Rutgers NJMS, Newark, NJ

Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability. TBI results in primary neuronal injury as well as secondary injury processes and inflammatory responses that contribute to long-term morbidity including epilepsy and memory deficits. Activation of the innate immune receptor, Toll-Like Receptor 4 (TLR4), plays a critical role in inflammatory

responses and increases risk for seizures after brain insults. In prior studies, we identified that an increase in TLR4 expression in hippocampal dentate neurons after fluid percussion injury (FPI) augments dentate AMPA currents through a mechanism independent of glia. Curiously, treatment with a TLR4 antagonist increased excitability and seizure susceptibility in sham controls without modulating glutamatergic currents. This study was conducted to examine TLR4 regulation of excitation /inhibition (E/I) balance in the uninjured and injured brain and identify the underlying mechanisms. Acute hippocampal slices were prepared from young adult Wistar rats, one week after lateral fluid percussion injury (FPI) or sham injury and naïve adult mice. In voltage-clamp recordings from dentate granule cells, TLR4 signaling enhanced afferent-evoked inhibitory postsynaptic current (eIPSC) amplitude in sham-controls while reducing eIPSC amplitude after FPI. Similarly, in granule cells from naïve mice, blocking TLR4 signaling in slices increased the E/I ratio of responses evoked by perforant path stimulation by selectively decreasing inhibition without altering excitation. A subset of FPI and sham rats received in vivo treatment with the TLR4 antagonist CLI-095 (0.5mg/kg, i.p) or vehicle one day after injury and underwent afferent-evoked local field potential (LFP) recordings in the dentate gyrus, in vivo under urethane anesthesia. CLI-095 treated sham rats showed enhanced dentate excitability in vivo compared to vehicle-treated controls (vehicle n=4, CLI-095 n=6 $p<0.001$). However, CLI-095 treatment attenuated increases in excitability observed after FPI (vehicle n=3, CLI-095 n=6 $p<0.05$). These data demonstrate that TLR4 signaling regulates dentate network excitability by enhancing inhibition in the uninjured and naïve brain while exacerbating excitability through a combination of changes in excitation and inhibition in the injured brain. These mechanistic differences in TLR4 signaling can be leveraged to limit epileptogenesis after brain injury.

Disclosures: **S. Nguyen:** None. **D. Subramanian:** None. **Y. Li:** None. **V. Santhakumar:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.08/G8

Topic: C.10. Brain Injury and Trauma

Support: MEXT JP16K18393

Title: MAPK-mediated phosphorylation of MKL2 regulates nuclear localization and transcriptional activity in striatal neurons

Authors: *A. ARIZA, Y. FUNAHASHI, K. KAIBUCHI;
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Abstract: Dopamine (DA) type 1 receptor (D1R) signaling activates cAMP/Protein kinase A, which then activates Mitogen-Activated Protein Kinase (MAPK) through Rap1 finally activating transcription factors (TFs) in striatal medium spiny neurons playing a pivotal role in reward-related behavior. How D1R signaling regulates transcription activity through phosphorylation remains unclear. In this study, we developed a proteomic analysis method using a KIX domain of CBP-binding protein (CBP-KIX) to identify TFs activated downstream D1R after cocaine administration. We identify 45 novel CBP interacting proteins in the mouse striatum including megakaryoblastic leukaemia-2 (MKL2) protein, a transcriptional co-activator of Serum Response Factor (SRF). We found that MAPK phosphorylates MKL2 at S913. We also found that cocaine exposure increased interaction of CBP and MKL2 in the nucleus accumbens in mice. MKL2 formed a ternary complex with CBP and SRF in vivo. Phosphorylation of MKL2 induces nuclear localization and easily increased cFos and NPAS4 promoter activity in striatal neurons. These results demonstrate that DA signaling regulates MKL2 nuclear localization and its interaction with CBP-KIX in a phosphorylation-dependent manner and thereby control SRF-dependent gene expression in striatal neurons.

Disclosures: A. Ariza: None. **Y. Funahashi:** None. **K. Kaibuchi:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.09/G9

Topic: C.10. Brain Injury and Trauma

Support: Air Force Medical Support Agency, U.S. Department of Defense, Program Project 308430 USUHS
Collaborative Health Initiative Research Program (NHLBI/NIH and USUHS)
U.S. Department of Defense in the Center for Neuroscience and Regenerative Medicine (CNRM)

Title: Microglia and vasculature in mouse models of repetitive mild head injury at high altitude

Authors: *K. WHITING[1,2], S. JAISWAL[3,4], F. W. LISCHKA[5], X. XU[2], C. WANG[7], G. YU[7], C. L. DALGARD[2,1], M. SZCZESNIAK[8], I. MAKALOWSKA[8], N. P. CRAMER[2], D. L. DICKSTEIN[6,4,1], D. P. PERL[6,4], B. J. DARDZINSKI[3,4,1], Z. GALDZICKI[2,4,1];
[1]Grad. Program in Neurosci., [2]Anatomy, Physiol. and Genet., [3]Radiology and Radiological Sci., [4]Ctr. for Neurosci. and Regenerative Med., [5]Biomed. Instrumentation Ctr., [6]Pathology, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [7]Computer Engin., Virginia Tech., Arlington, VA; [8]Mol. Biol. and Biotech., Adam Mickiewicz Univ., Poznan, Poland

Abstract: Repetitive mild traumatic brain injury (rmTBI) is a significant health concern, often causing disability and progressive neurological deficits. Physiological response to injury is influenced by environmental factors, such as high altitude exposure, which is characterized by a reduction in available oxygen for absorption by blood and tissue through hypobaric hypoxia (HH). This has serious implications for cognitive, cardiovascular, and metabolic functioning, and may exacerbate the severity of rmTBI. Athletes, climbers and military service members operating in the mountains are at serious risk for HH related complications following TBI. Understanding the mechanisms behind environment and injury recovery interactions is critical for appropriate clinical considerations and preventative care. Our previous research shows altered angiogenesis pathways and increased microglial phagocytosis following HH and altered hippocampal circuitry after rmTBI. We have also seen functional changes in hippocampal mediated behavior in mouse models, as well as HH induced alterations in glucose uptake. Here we show that alterations in microglial activity and vascularization drive responses to environmental HH and brain injury. Our studies on HH exposure and TBI indicated changes to white matter function, prompting our measurement of compound action potentials (APs) in corpus callosum of combined rmTBI/HH models. Our preliminary electrophysiological data measured normal AP velocities, but neuronal excitability was altered. MicroCT has revealed abnormal neurovascular structure following HH which may account for HH induced neurovascular pathology. Transcriptional analysis of hippocampal and cortical microglia following HH exposure and rmTBI administration suggests potential roles of inflammation in neurovascular adaptive responses. These transcriptional changes led us to investigate microglia chemotaxis related to neurovascular injury in our previously developed Cx3Cr1-GFP mice for *ex vivo* 2-photon imaging following HH exposure or rmTBI. Transcriptional profiles and immunohistochemical analysis of vascular fractions isolated from mouse brains examine the intricacies of these structural changes and functional interactions. Future research will focus on how microglia modulate these neurovascular changes, and how the influence of metabolic alterations may impact adaptation and recovery.

Disclosures: K. Whiting: None. **S. Jaiswal:** None. **F.W. Lischka:** None. **X. Xu:** None. **C. Wang:** None. **G. Yu:** None. **C.L. Dalgard:** None. **M. Szczesniak:** None. **I. Makalowska:** None. **N.P. Cramer:** None. **D.L. Dickstein:** None. **D.P. Perl:** None. **B.J. Dardzinski:** None. **Z. Galdzicki:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.10/G10

Topic: C.10. Brain Injury and Trauma

Support: NS077675

Title: Optogenetically-identified alterations in synaptic efficacy of parvalbumin interneurons after traumatic brain injury

Authors: *A. C. HARRIS, Jr, K. M. JACOBS;
Virginia Commonwealth Univ., Richmond, VA

Abstract: Alterations in the balance of synaptic excitation and inhibition have been reported in diverse experimental models of traumatic brain injury (TBI). Recently our group has published histological studies demonstrating a reduction in $GAD67^+$/$parvalbumin^+$ (PV) synaptic boutons onto the perisomatic region of layer 5 pyramidal neurons after mild TBI (mTBI) (Vascak 2017). To assess the specific functional consequences of mTBI on the cortical network of PV, fast-spiking (FS) interneurons, we took advantage of optogenetics to control selectively the output of this neuronal population. We randomized PV-Cre;Ai32(RCL-ChR2(H134R)/EYFP male mice, which express channelrhodopsin-2 (ChR2) selectively in PV/FS interneurons, to receive either sham surgery or a central fluid percussion injury, an experimental model of diffuse mTBI (1.64±0.1 atm severity) at 6-8 weeks of age. Patch clamp recordings were made in ex vivo slices 1 day after surgery from either $EYFP^+$ PV/FS interneurons or pyramidal neurons within layer 5 in the region of the injury or homotopic sham somatosensory cortex. Blue light of varying durations passed through the objective above the recorded neuron was used to activate the PV/FS population. ChR2 was equally effective in depolarizing the PV/FS population in sham and mTBI. PV/FS interneurons had similar intrinsic and membrane properties in sham and mTBI groups, including on measures of rheobase, action potential (AP) half-width, AP height, AP maximum frequency, and the slope of the input-output curve. Compared to sham animals, light-induced, PV/FS interneuron-specific IPSCs in layer 5 pyramidal neurons from mTBI mice were reduced in amplitude at brief durations of blue light (0.08 - 0.16 msec; n = 10 and 16 cells from 4 and 5 mice for sham and mTBI, respectively). Surprisingly, at longer durations of blue light, sham and mTBI were not different, while at the longest durations the IPSC amplitude in mTBI surpassed that of the sham condition (n = 17 and 11 cells from 7 and 7 mice for sham and mTBI, respectively). The results of the present study indicate disruption of the synaptic dynamics of the PV/FS interneuron population in mTBI. This disruption may translate to aberrations in the entrainment of the coherent network rhythms underlying cognition.

Disclosures: **A.C. Harris:** None. **K.M. Jacobs:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.11/G11

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 5R37NS077908-07

Title: Neurotoxicity, neuroprotection, and the visibility of dying neurons before microglial engulfment

Authors: *T. BALENA[1], N. RAHMATI[4], K. P. LILLIS[2], K. J. STALEY[3];
[2]Neurol., [1]Massachusetts Gen. Hosp., Charlestown, MA; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Harvard Med. School/Massachusetts Gen. Hos, Boston, MA

Abstract: Neuronal death terminates with microglial engulfment of apoptotic neurons or necrotic neuronal debris. At present estimates of neuronal death are based on the number of neurons that are visibly undergoing the death process. This number is a function of three variables: the rate at which neurons are entering the death process; the visibility of neurons during the process (which depends largely on the membrane permeability delimiting the rate of biomarker uptake); and the rate at which they are exiting the process via microglial engulfment. Organotypic hippocampal slice cultures were made from P6 wild-type C57BL/6J mice, and sequential two-photon imaging was performed using transgenic fluorophores as well as the ratiometric Na^+ dye SBFI-AM. Neurons entering the death process quenched fluorescent proteins (such as TurboRFP) concurrent with elevated caspase activity (visualized with FLICA). Due to their increased membrane permeability (visualized with Annexin V and PO-PRO-1) these neurons rapidly took up AM ester dyes such as SBFI, lost their neuronal processes, and exhibited evidence of DNA degradation (as seen with chromatin staining). The mitochondria and membrane transporters of dying neurons remained active for as long as two weeks after entering this pathway. Throughout this period, the permeability of the neuronal membranes gradually increased, as evidenced by a progressive increase in intracellular sodium concentrations ($[Na^+]_i$), and eventually reached the point where polar dyes such as propidium iodide (PI) entered the nucleus. The death process ended with microglial engulfment. The three variables were then studied during exposure to conditions in the developing nervous system considered to be neurotoxic (ethanol) or neuroprotective (kynurenate and tetrodotoxin). Ethanol did not affect fluorescent protein quenching but did alter $[Na^+]_i$, whereas kynurenate and tetrodotoxin did not affect $[Na^+]_i$ but did alter PI positivity and microglial engulfment. We conclude that neuronal death is delayed in the developing hippocampus after brain injury, and that current assays of neuronal death are misleading in this setting. Sequential assays of cellular fluorescent protein expression provide the most robust measure of changes in the number of viable neurons.

Disclosures: **T. Balena:** None. **N. Rahmati:** None. **K.P. Lillis:** None. **K.J. Staley:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.12/G12

Topic: C.10. Brain Injury and Trauma

Support: NIH grant RO1 NS101955

Title: Contribution of injury-induced hippocampal neurogenesis to cellular learning and memory function

Authors: *N. M. WESTON, A. T. ROLFE, T. M. REEVES, D. SUN;
Anat. and Neurobio., Virginia Commonwealth Univ., Richmond, VA

Abstract: The hippocampus is vulnerable to traumatic brain injury (TBI), its damage is related to cognitive deficits that are often the hallmark of TBI. Recent studies have found that TBI induces enhanced neurogenesis in the dentate gyrus (DG) of the hippocampus and it is related to innate cognitive recovery. However, mechanisms of DG neurogenesis contributing to post-TBI recovery remain unclear. This study investigated changes of long-term potentiation (LTP) within the DG in association to TBI-induced neurogenesis. Adult male rats received a moderate TBI or sham injury and were sacrificed for brain slice recordings at 30d or 60d post-injury. Recordings were taken from the medial perforant path input coming from the entorhinal cortex to the dentate granule cells in the presence or absence of the GABAergic antagonist picrotoxin. The presence of picrotoxin in the bath solution reflects the activity of total DG granule cells including both mature and newborn cells. In contrast, the absence of picrotoxin reflects predominately the activity of newborn granule cells, due to their relative insensitivity to GABAergic inhibition. During recording sessions, fEPSPs were evoked using perforant path stimulation at a rate of 1/30 sec. After establishing a stable fEPSP baseline, LTP was induced using 100 Hz stimulation. Post-LTP recordingcontinued for 1 hour. Measurements of LTP observed in the total granule cell population (with picrotoxin) showed a prolonged LTP impairment which worsened between 30 and 60 days post-TBI. ANOVA analyses revealed significant overall differences between groups [$p<0.05$], and posthoc comparison showed significantly lower LTP evoked at 60d post-TBI, than that in sham rats ($p<0.05$). The magnitude of LTP observed at 30d was not significantly different from shams, but the mean level was intermediate between sham and the 60d. Under conditions which predominantly reflected the LTP elicited in newly born granule cells (no picrotoxin), a strikingly different pattern of post-TBI changes was observed, with a time-dependent cycle of functional impairment and recovery observed. ANOVA analyses revealed significant overall differences between groups [$p<0.05$]. At 30d post-injury this cell population showed little or no LTP. Posthoc comparisons showed that by 60d the capacity for LTP in the newly born granule cells increased to a level equal or above that of sham controls. The magnitude of LTP at 60d was significantly greater than that observed at 30d post-TBI ($p<0.05$). The results demonstrate a remarkable capacity for newly born granule cells to mount a recovery response and participate in functional changes that may be essential for cognitive recovery post-TBI.

Disclosures: **N.M. Weston:** None. **A.T. Rolfe:** None. **T.M. Reeves:** None. **D. Sun:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.13/G13

Topic: C.10. Brain Injury and Trauma

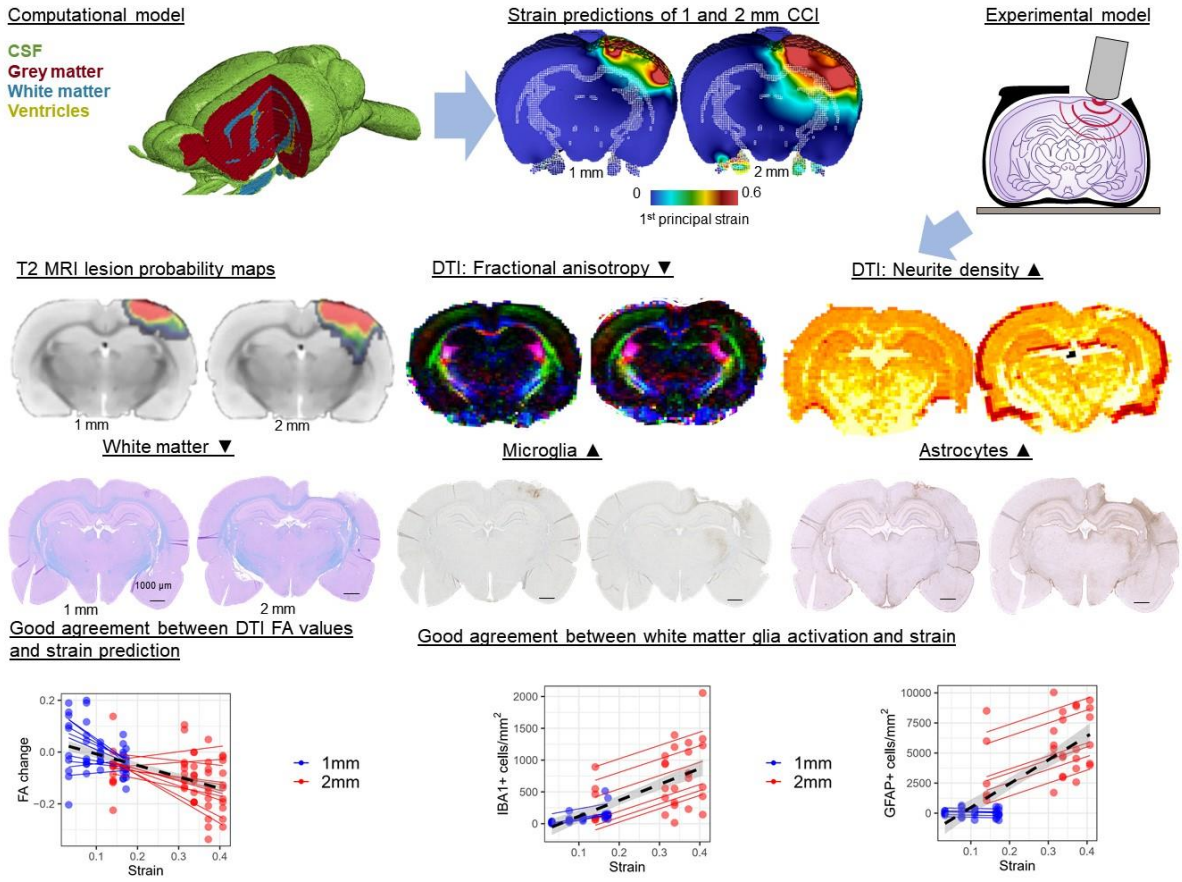
Support: ISSF

Title: Finite element modelling strain prediction of a rat impact injury model correlates with measures of MRI and pathophysiology

Authors: C. K. DONAT[1], M. GHAJARI[2], M. YANEZ LOPEZ[2], M. GOLDFINGER[2], N. BAXAN[2], R. SEEAMBER[2], J. CHADWICK[2], F. MUELLER[2], P. SIEGKAS[2], S. M. GENTLEMAN[3], *M. SASTRE[2], D. J. SHARP[2];
[1]Div. of Brain Sci., [3]Med., [2]Imperial Col. London, London, United Kingdom

Abstract: Traumatic brain injury causes a substantial global disease burden. Transfers of large biomechanical forces during injury cause varying strain rates dependent on tissue properties. However, the relationship between biomechanical forces and pathology, especially in the white matter, is still poorly understood. We used a high-fidelity finite element (FE) model of the rat brain to test whether strain predictions correspond to MRI and quantitative histopathology measures.
An atlas of the rat brain was used to develop a finite element model, incorporating detailed anatomy and material models for nonlinear impact simulations. Using a Controlled Cortical model, we performed 1 and 2 mm impacts in anaesthethized male rats (n=21). Animals received 9.4 T MRI (3D T1 and T2, multi-shell diffusion tensor imaging), pre- and 14 days post-injury. On day 15, animals were euthanized and brains processed for quantitative histopathology of white matter thickness (Luxol Fast Blue, LFB) and astro- and microglial response (GFAP/IBA1). The FE model yielded large strains in the impacted cortex and deeper structures, including corpus callosum (CC). In the cortex, FE prediction showed a good overlap with T2 lesion probability maps. Patterns of tissue loss corresponded to T2 and strain prediction maps.
In the CC, DTI showed a significant decrease of fractional anisotropy (FA) in the 2 mm injured animals (-17%) as compared to baseline. A mixed-model of DTI measures and strain predictions showed that both strain and injury severity affected FA, neurite density and orientation dispersion, the latter were found to be significantly increased (2 mm, 57% and 14%).
Using quantitative histopathology we saw a significant thinning of LFB-stained CC (1 mm: -9%; 2 mm: -24%) and significantly increased numbers of both microglia and astrocytes (between 1-4 fold), again correlating with strain predictions.
Our data indicates that computational strain calculations allow the prediction of major areas of

tissue damage, supported by a good agreement between patterns of biomechanical forces and sub-acute brain damage.



Disclosures: **C.K. Donat:** None. **M. Ghajari:** None. **M. Yanez Lopez:** None. **M. Goldfinger:** None. **N. Baxan:** None. **R. Seeamber:** None. **J. Chadwick:** None. **F. Mueller:** None. **P. Siegkas:** None. **S.M. Gentleman:** None. **M. Sastre:** None. **D.J. Sharp:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.14/G14

Topic: C.10. Brain Injury and Trauma

Support: The Methodist Hospitals Endowed Professorship in Neuroscience (AR)
NIH grant NS-081370 (AR)

The University of Tennessee Neuroscience Institute (CD)
Dunagan Medical Education Fund (AM)
TBI Research Fund at UTHSC

Title: Progressive long-term spatial memory loss following repeat concussive and subconcussive brain injury in mice is associated with hippocampal neuron loss and altered microglial phenotypes

Authors: A. REINER, N. A. DEL MAR, C. C. DORIAN, J. D. WORTHEN, A. C. MICETICH, ***M. G. HONIG**;
Anat. & Neurobio., The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: The means by which repeat brain trauma initiates long-term pathogenic processes leading to memory loss is uncertain. To model repeat head trauma, we delivered multiple air blasts (4 blasts, spaced a week apart), to the left side of the cranium of otherwise shielded and head-stabilized mice. We used two pressure amplitudes for the blasts: 50-psi, which causes widespread axonal injury and a variety of functional deficits when delivered singly, and 30-psi, which yields only minor neuropathology and no significant functional impairment when delivered singly, henceforth called concussive and subconcussive, respectively. The repeat blast mice, in comparison to a control group of mice that received repeated 0-psi (sham) blast, showed no obvious memory loss at 4 months, but did exhibit deficits in spatial memory (assessed by contextual fear testing and by spontaneous alternation in an X-maze) at 14 months post-blast. Histological analyses showed neuron loss in dentate and CA1 of dorsal hippocampus, and a reduction in neurogenesis in dentate. Surprisingly, spatial memory deficits and neuron loss were greater in the subconcussive mice, and the contrecoup right side showed more neuron loss and less neurogenesis than did the left side for both concussive and subconcussive mice. We then explored the possible role of microglia in the emergence of long-term memory loss and dorsal hippocampal pathology by immunostaining for four microglial proteins (MHCII, CX3CR1, CD68 and IBA1) to assess expression levels and using the IBA1 labeling to measure morphometric traits. We found significant differences between experimental groups and right vs left hippocampus for several parameters. Hierarchical cluster analysis revealed five distinct microglial types across the total set of >400 cells. Two of these types were more prevalent in sham hippocampus and their prevalence was linked to better neuron survival and spatial memory (1A and 1B), while two other types (2B and 2C) were more prevalent in repeat blast hippocampus and were linked to poorer neuron survival and spatial memory. Types 2B and 2C had low expression of MHCII, CX3CR1, CD68 and IBA1 and/or attenuated processes, suggesting their functional capacity may have been diminished. In addition, microglia were less abundant in right hippocampus of repeat subconcussive mice than in repeat concussive and sham. Taken together, our findings suggest that both repeat concussive and repeat subconcussive head injury have an effect on microglia that ultimately leads to a decreased abundance of beneficial microglia and an increase in nonbeneficial microglia, which may in turn drive progressive neuronal degeneration.

Disclosures: **M.G. Honig:** None. **A. Reiner:** None. **N.A. Del Mar:** None. **C.C. Dorian:** None. **J.D. Worthen:** None. **A.C. Micetich:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.15/G15

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 5-R01-NS040109-19

Title: Brain extracellular matrix alters local ion concentrations and responses to injury

Authors: *K. P. NORMOYLE[1], V. I. DZHALA[2], K. P. LILLIS[3], K. EGAWA[5], J. C. GLYKYS[6], N. RAHMATI[7], K. J. STALEY[4];
[1]Neurology/Child Neurol., Massachusetts Gen. Hosp., Boston, MA; [2]Dept. of Neurol., [3]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [4]Massachusetts Gen. Hosp., Boston, MA; [5]Dept. of Pediatrics, Hokkaido Univ. Grad. Sch. of Med., Sapporo, Japan; [6]Dept. of Pediatrics, Univ. of Iowa. Carver Col. of Med., Iowa City, IA; [7]Harvard Med. School/Massachusetts Gen. Hos, Boston, MA

Abstract: The chloride concentration immediately surrounding neurons is canonically considered to equal the chloride in the bulk cerebrospinal fluid. However, neurons are surrounded by an extracellular matrix comprised largely of heavily sulfated glycosaminoglycans. We first tested whether the sulfate moieties change the local chloride concentration. Using Fluorescence Lifetime IMaging (FLIM) of chloride-sensitive fluorophores constrained to the extracellular space by conjugation with 10 kilodalton dextran, we found that the extracellular chloride ($[Cl^-]_o$) between neurons in the depths of acute hippocampal slices and at all depths of organotypic hippocampal slice cultures was only half of the bulk CSF chloride. Freeing fixed sulfate moieties by partial dissolution of the matrix using Chondroitinase ABC increased $[Cl^-]_o$. These results confirm earlier observations using commercially available chloride-sensitive dyes such as MEQ.
We next asked what would happen to $[Cl^-]_o$ if the sulfate moieties of the matrix were freed by endogenous matrix metalloproteinases (MMPs), which are freed and activated after brain injury. Using acute brain slices as well as 2-photon photolysis of single neurons within organotypic slices as models of brain injury, we found a strong dependence of $[Cl^-]_o$ vs distance from injury, with concentration increasing to the ACSF levels near the injured surface of acute slices or proximity to photolysed neurons in organotypic slices. These changes in $[Cl^-]_o$ should alter the neuronal intracellular chloride via the activity of the high-velocity equilibrative membrane chloride transporters. We have previously reported such changes in slices and confirmed them in both injury models here. If the injury-induced increases in extra- and intracellular chloride were due to release of extracellular sulfates and replacement by chloride, these sulfates should be released to the perfusate, and we confirmed this using colorimetric assays of chondroitin sulfate.

Finally, the release of sulfates should be inhibited by MMP antagonists, and we confirmed that broad-spectrum inhibition using the zinc chelator ZX-1 or the more specific MMP-2/9 inhibitor SB3CT also reduced the extracellular and intracellular chloride concentration and neuronal volume at the surface of cut slices and in proximity to photolysed neurons.
We conclude that $[Cl^-]_o$ is partially displaced by sulfates in the extracellular matrix, and that damage to the extracellular matrix following brain injury alters the distribution of chloride in both the extra- and intracellular spaces. These findings have immediate implications for the treatment of cytotoxic edema after brain injury.

Disclosures: K.P. Normoyle: None. **K.J. Staley:** None. **V.I. Dzhala:** None. **K.P. Lillis:** None. **J.C. Glykys:** None. **N. Rahmati:** None. **K. Egawa:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.16/G16

Topic: C.10. Brain Injury and Trauma

Support: Department of Defense (W81XWH-12-1-0536)

Title: Carbamates and organophosphates, differential effects on axonal transport

Authors: *S. X. NAUGHTON[1], A. V. TERRY, JR[2];
[1]Pharmacol., [2]Dept Pharmacol Toxicol, Augusta Univ., Augusta, GA

Abstract: Organophosphate (OP) and carbamate-based chemicals are used effectively worldwide as pesticides to improve farming productivity and to combat vector borne illnesses. However, such wide spread use has become an environmental concern, since a variety of long-term neurological symptoms have been associated with repeated exposures to these compounds. One case where a large number of people are known to have been exposed to both OPs and carbamates is the first gulf war where aggressive spraying was conducted to control insects. In addition, up to 250,000 soldiers received the carbamate pyridostigmine bromide as a nerve agent pretreatment. In epidemiological studies, 26-32% of gulf war soldiers have chronic health problems including fatigue, memory deficits, and mood alterations, symptoms collectively known as "Gulf War Illness"(GWI). Importantly, both carbamates and OPs have been implicated in the symptoms of GWI. The mechanistic basis of GWI is unknown, however, since it was not typical for soldiers to exhibit classic cholinergic-based symptoms that would normally be associated with acetylcholinesterase (AChE) inhibition after acute toxic exposures to OPs or carbamates. Animal studies in our laboratory have indicated that repeated exposures to OPs, below the threshold for acute toxicity can result in learning and memory deficits, which resemble those observed in GWI. Moreover, we have identified OP-related axonal transport (AXT)

deficits as a potential non-cholinesterase mechanism for the learning and memory deficits. To date, we have not evaluated carbamates. Interestingly, the carbamates pyridostigmine bromide and rivastigmine have been used therapeutically for many years for myasthenia gravis and Alzheimer's disease, respectively, and to our knowledge GWI-like symptoms have not been observed in these patients. Accordingly, the purpose of the experiments described here was to evaluate the carbamate physostigmine (a highly potent AChEI) on AXT using an *in vitro* live imaging assay that we have previously found to be very sensitive to OP-related deficits in AXT. An additional objective was to evaluate the OP tribufos (DEF) a cotton defoliant that reacts very weakly with AChE. While we have observed OP-related AXT deficits *in vitro* well below the threshold for AChE inhibition, it is expected that the results of the two studies described here would help us determine if the AXT deficits are unique to OP-based AChEIs and whether OPs that are not potent AChEIs also impair AXT. The results of our experiments with physostigmine (24 hr exposure) across a wide range of concentrations indicated no impairments of AXT. The experiments with DEF are ongoing.

Disclosures: S.X. Naughton: None. **A.V. Terry:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.17/G17

Topic: C.10. Brain Injury and Trauma

Support: NIH R01 NS106925
PA Department of Health grant 4100077083

Title: Reduction of neurogranin protein expression after controlled cortical impact

Authors: *S. SVIRSKY[1], S. W. CARLSON[2], J. HENCHIR[2], C. E. DIXON[2,3];
[1]Ctr. for Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Neurosurg., Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA; [3]VA Pittsburgh Healthcare Syst., Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) is known to cause short and long-term synaptic changes in the brain and these processes underlie downstream cognitive and behavioral impairments. Neuronal levels of neurogranin, a calcium-sensitive calmodulin-binding protein essential for synaptic plasticity and postsynaptic signaling, are tightly correlated with cognitive function. Thus, it has been established as a synaptic biomarker for Alzheimer's disease and, more recently, TBI. This study aims to understand the role of neurogranin in synaptic dysfunction after TBI, by characterizing changes in protein expression between 24 hour and 2-week time points. Under isoflurane anesthesia, adult male Sprague Dawley rats (275-300g) were subjected to either controlled cortical impact to simulate a moderate-to-severe TBI (2.8mm deformation, 4m/s) or

control surgery. Expression of neurogranin was evaluated in the cortex and hippocampus by western blot and immunohistochemistry at 24 hour, 1-week and 2-week time points post-injury. Student t-tests were conducted for each region compared to control. At 24hrs, distinct regional changes in neurogranin levels were observed. Protein expression was significantly reduced in both the ipsilateral and contralateral hippocampus ($p=0.0095$; $p=0.0087$), however no change was observed in cortex. At 1 week, injury proximity drove changes in neurogranin. Lower protein levels were observed in the ipsilateral hippocampus and cortex ($p=0.0043$; $p=0.0022$), while the contralateral hemisphere showed no changes. The same expression pattern was observed 2 weeks post-injury ($p=0.0381$; $p=0.0411$). Qualitative immunohistochemical assessment corroborated our western blot findings. Particularly, the peri-contusional cortex and hippocampal CA3 region showed marked reduction in immunoreactivity. Our results indicate that controlled cortical impact lowers neurogranin expression with both temporal and regional specificity. The ipsilateral cortex and hippocampus were especially vulnerable at all time-points, as compared to the contralateral hemisphere. This is the first study, to our knowledge, to investigate changes in neurogranin over time, in the brain using a controlled cortical impact rodent model of traumatic brain injury. Further understanding of changes in synaptic biology allows us to further elucidate pathological mechanisms contributing to the cognitive and behavioral dysfunction after injury.

Disclosures: **S. Svirsky:** None. **S.W. Carlson:** None. **J. Henchir:** None. **C.E. Dixon:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.18/G18

Topic: C.10. Brain Injury and Trauma

Support: NSF CAREER 1349735
NIH R01 AR063712
T32 GM-065103

Title: Nuclear responses in neural cell cultures after applied impulse strain results in chromatin condensation

Authors: *S. E. SCHNEIDER[1], B. SEELBINDER[1], S. GHOSH[1], R. L. WILSON[1], D. M. PIERCE[2], C. P. NEU[1];
[1]Univ. of Colorado Boulder, Boulder, CO; [2]Univ. of Connecticut, Storrs, CT

Abstract: Traumatic Brain Injury (TBI) is a significant health crisis affecting all genders, age groups, and nationalities. From initial impact (caused by mechanical stress) to subsequent tissue deterioration (caused by mechanical and biochemical stresses), the central nervous system is

working to maintain its environment and return to its homeostatic state. Disruptions in within its environment range from systemic inflammation to internal cytoskeletal damage. Mechanical information relayed from the cell's internal environment has been shown to direct cellular responses. In the pathogenesis of TBI, information examining mechanical-to-molecular thresholds could help explain the biomolecular and biophysical perturbations experienced after injury. To further examine the mechanical-to-molecular thresholds, we derived mixed co-cortical cells from E18.5 H2B-eGFP mice. Co-cultures were plated on soft PDMS (~5kPa) coated stretchable membranes in custom-made wells. On day 14 of *in vitro* culture, cells were subjected to mechanical insult, uniaxial strain, using a custom-built pneumatic device mounted on the stage of a Nikon A1R confocal microscope. In the well, nuclei of cells within a 0.68 cm^2 area were examined for biophysical responses to mechanical insult. Strain magnitudes within the area of mechanical insult ranged from -8% to 30%. The local strain value influencing the nucleus was calibrated using a finite analysis model. Images were adjusted for translation drift, and nuclei were classified into two groups based on observed whole nuclear rotation and internal chromatin motion. Rotational nuclei were further analyzed prior to and after mechanical insult to characterize biophysical features. Neural cells *in vitro* demonstrated differences in nuclear responses after a single applied mechanical insult between the different strain magnitudes. With increasing strain magnitudes, cell nuclei transitioned from dominant internal chromatin motion to increased rotational motion. Further analysis of rotational nuclei demonstrated alterations in chromatin condensation within the nuclei after applied strain. This data suggests divergent nuclear mechanosensitive responses of cells in the mechanically protected environment of the skull. We expect that distinct biophysical features will be enhanced with repetitive loading models, and plan to use this analysis to further define biomolecular features at defined strain magnitudes.

Disclosures: S.E. Schneider: None. **B. Seelbinder:** None. **S. Ghosh:** None. **R.L. Wilson:** None. **D.M. Pierce:** None. **C.P. Neu:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.19/G19

Topic: C.10. Brain Injury and Trauma

Title: Persistent upregulation of hippocampal synaptic transmission and altered Ca^{2+} handling following single or repeated closed head concussive impacts

Authors: *J. MCDAID[1], C. A. BRIGGS[1], A. LITTLEFIELD[1], N. M. BARRINGTON[1], D. A. PETERSON[2], D. A. KOZLOWSKI[3], G. E. STUTZMANN[1];
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Abstract: Traumatic Brain Injury (TBI) affects 1.7 million people annually and is a widespread clinical concern with long-term detrimental neurological and behavioral symptoms. TBI that doesn't result in obvious cell death or massive tissue damage is typically called mild TBI (mTBI), or concussion. Recently, much attention has been given to discriminating single concussion from repeated concussion deficits, with repeated concussion leading to worsened clinical symptoms, including memory loss and impaired thinking, which may be due to changes in hippocampal synaptic transmission. To assess the long-term effects of single or repeated concussive impacts on mediators of memory encoding such as synaptic transmission and plasticity, and cellular $^{Ca2+}$ signaling, we utilized a novel closed-head controlled cortical impact (CCI), a recently optimized approach which closely replicates the mode of injury in clinical cases. We also utilized a prolonged period of 30 days post-CCI to examine persistent injury effects. Long Evans rats (under anesthesia) received a sham procedure or a head impact at a location overlying the sensorimotor cortex, using a modified Leica CCI device. Experimental groups received a single impact or three successive impacts, separated by 48 hour intervals. After 30 days, hippocampal slices were prepared for field recordings and whole-cell patch recording/2-photon $^{Ca2+}$ imaging. Field recordings were performed in the CA1 stratum radiatum using stimulation of CA3-CA1 Schaffer collaterals, with whole-cell patch/2-photon recordings performed on CA1 pyramidal neurons. Field recordings consisted of input-output functions and LTP induction. In both the single and repeated concussion rats, slices had significantly increased synaptic responses when compared to sham animals, indicating a sustained hyper-excitability in neuronal circuitry. This synaptic potentiation was not accompanied by significant effects on LTP expression, but basal $^{Ca2+}$ and voltage-gated $^{Ca2+}$ signals were elevated in both concussion groups, and ryanodine receptor-mediated $^{Ca2+}$ responses through ER stores were significantly decreased in the repeated concussion group only. In addition, action potential activation threshold was elevated in the single concussion group, indicating possible decreased somatic membrane excitability. Thus, single and repeated concussion may lead to a similarly persistent upregulation of select excitatory hippocampal synapses, possibly through changes in postsynaptic $^{Ca2+}$ signaling/regulation, and these effects may contribute to the more detrimental long-term cognitive symptoms seen in both single and repeated concussion patients.

Disclosures: J. McDaid: None. **C.A. Briggs:** None. **A. Littlefield:** None. **N.M. Barrington:** None. **D.A. Peterson:** None. **D.A. Kozlowski:** None. **G.E. Stutzmann:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.20/G20

Topic: C.10. Brain Injury and Trauma

Title: EMT snail regulates astrocyte polarity after cortical lesions in the adult CNS

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Abstract: Epithelial-Mesenchymal Transition (EMT) is a phenotypic plasticity by which epithelial cells transdifferentiate into mesenchymal cells becoming an essential process for embryonic development, cancer and, fibrosis progression. Transitions in cell polarity states enable EMT and comprise a set of distinct asymmetric distribution of cellular constituents that are fashioned to allow specialized cellular functions including cell migration and fate determination. Activation of the transcription factor Snail is a critical step for EMT and in the adult this zinc finger protein disrupts tissue homeostasis triggering a pathological transition. Furthermore, Snail appears to be required for the majority of EMT types and it is a direct transcriptional repressor or E-cadherin. The repression of E-cadherin is a hallmark of EMT that through downregulation of initiating signaling events and cytoskeletal arrangements switches the cell apical-basal polarity to a frontal-rear polarity state. It is unknown whether this rearrangement is induced in the adult CNS in response to injury and which polarity mechanisms in subsequent scar formation are directly regulated by Snail expression affecting brain recovery after lesion. In this study, we have examined *in vivo* the role of the EMT triggered by Snail after 7 days following a cortical lesion in the CNS of adult mice. We characterized these responses using a combination of a cortical wound model, immunohistochemistry/3D morphological analysis and antisense oligonucleotides (ASOs) technology to silence the expression of Snail. Our results suggest EMT is triggered in the adult CNS after a cortical lesion and this response appears to be orchestrated through the expression of Snail in a specific population of cells in the area of the lesion. Dynamic remodeling in astrocyte morphology is critical in CNS injury responses and our data suggest that EMT signaling pathways are important in modulating these astrocytic changes. We propose that the EMT pathway contributes to glial scar formation following CNS injury.

Disclosures: C.C. Clarkson: None. **M. Karl:** None. **R.H. Miller:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.21/G21

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 2R56AG025970-11A1

Title: Regulation of BACE1 expression after injury is linked to the p75 neurotrophin receptor

Authors: *K. SAADIPOUR, M. V. CHAO;
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Abstract: BACE1 is a transmembrane aspartic proteinase that cleaves many substrates, and are required for normal brain function. BACE1 expression is high during development, but it is reduced during adulthood. Under conditions of stress and injury, the level of BACE1 become enhanced; however, the exact mechanisms that drive BACE1 expression under these circumstances are not well understood. Here we discovered that the expression of BACE1 in cortex is elevated following controlled cortical impact (CCI) and focal cortical injury in male B6SJL mice. One mechanism associated with brain injury is activation of potentially injurious p75 neurotrophin receptor (p75), which can trigger pathological signal transduction. In this study, we found that both BACE1 and p75 are highly expressed after traumatic brain injury in male B6SJL mouse cortex. Unexpectedly, the two proteins are also tightly co-expressed in cortical areas after damage. Genetic deletion or transduction of p75 in mouse primary cultured neurons and cell lines resulted in concomitant effects upon BACE1 levels. Reporter gene assays BACE1 promoter showed that p75 expression was closely associated with the increase in enhancement of BACE1 expression. Furthermore, our data implicate that the c-jun n-terminal kinase (JNK), a p75 downstream substrate, contributes to the activation of BACE1 expression. Finally, cleavage of amyloid precursor protein (APP) by BACE1 was increased in the presence of p75 receptors in neuronal and cell culture. This study illustrates that two injury-induced molecules are intimately connected and suggests a potential link between p75 signaling and the expression and activity of BACE1 after brain injury.Keywords: BACE1, p75 neurotrophin receptor, JNK, traumatic brain injury

Disclosures: M.V. Chao: None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.22/G22

Topic: C.10. Brain Injury and Trauma

Support: NIH-NIA P30AG013846
Private Foundation

Title: Imaging blood-brain barrier (BBB) disruption and traumatic microvascular injury by DCE-MRI and LA-ICP-MS

Authors: *L. E. GOLDSTEIN[1], O. MINAEVA[2], N. HUA[2], N. LIPOLI[2], E. S. FRANZ[3], C. A. TAGGE[3], A. M. FISHER[3], R. VEKSLER[4], X. LIU[2], S. E. RIND[2], K. BABCOCK[2], J. A. MONCASTER[2], A. FRIEDMAN[5], A. C. MCKEE[2];
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Abstract: Traumatic brain injury (TBI) is associated with significant secondary morbidity, including increased risk of late-life cognitive impairment and age-related neurodegenerative disease such as chronic traumatic encephalopathy (CTE). In this study, we evaluated *in vivo* dynamic contrast-enhanced MRI (DCE-MRI) with gadofosveset trisodium (a gadolinium-based contrast agent that binds serum albumin) to detect, localize, and track traumatic microvascular injury (TMI) and blood-brain barrier (BBB) dysfunction in mice after lateral closed-head concussive impact injury. We used laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to map spatial distribution of Gd in mouse brains harvested 2 weeks post-injury to confirm that detected DCE-MRI abnormalities represented true BBB permeability dysfunction. Gd maps of brains obtained from impact-injured mice revealed enhanced Gd accumulation that colocalized with T1-weighted hyperintensities and BBB disruption detected by DCE-MRI as well as focal deposition of serum albumin in the brain parenchyma. These findings were observed without evidence of brain hemorrhage. We also investigated spatial distribution of gadolinium deposition in cerebral cortex in humans with and without history of traumatic brain injury after repeated exposure to intravenous Gd-containing contrast agents. Non-homogeneous gadolinium retention was detected by LA-ICP-MS imaging in cerebral cortex, pia mater, and pial-ensheathed leptomeningeal blood vessels from human subjects with normal renal function and history of traumatic brain injury. Our results provide “proof of concept” feasibility validation of using DCE-MRI *in vivo* for diagnostic evaluation of BBB dysfunction in the acute-subacute period after closed-head impact injury and demonstrate the utility of *in vivo* DCE-MRI and *ex vivo* LA-ICP-MS to localize and quantitate non-hemorrhagic BBB disruption post-injury.

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Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.23/G23

Topic: C.10. Brain Injury and Trauma

Title: The neurobehavioral and neuropathological effects of a novel experimental model of repeated mild traumatic brain injury

Authors: *S. J. MCDONALD[1], L. PHAM[2], W. T. O'BRIEN[1], D. K. WRIGHT[1], S. R. SHULTZ[1];
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Abstract: BACKGROUND: Although there is some evidence that exposure to multiple mTBIs may lead to lingering or even chronic impairments, more research is required to understand these associations and the underlying mechanisms. In attempt to further understanding, we have developed a clinically relevant, awake closed head injury (ACHI) model in rats. We have recently shown that a single ACHI induces transient neurobehavioral and glial disturbances. In this study, we aimed to determine the short- and long-term neurobehavioral and neuropathological effects of repeated ACHIs.
METHODS: Adolescent male rats were placed in a restraint bag and a 3D printed steel helmet positioned over the head such that the impact target was centred over the left parietal bone. Once positioned on a foam platform, an electromagnetic impactor was used to strike the helmet. Sham animals underwent the same procedure without impact. Rats were given four sham or four ACHI procedures (inter-injury interval of 48h) and allocated to either 1-week or 3-month recovery groups. Behavioural analysis included assessments of sensorimotor ability (beam task), anxiety (open field and elevated plus maze) and cognitive function (Y-maze and Morris water maze). Neuropathological analysis included quantification of several markers associated with neuroinflammation and neurodegeneration, as well as *ex vivo* diffusion weighted imaging to assess white matter integrity.
RESULTS: Preliminary findings revealed that rats given four ACHIs had sensorimotor and cognitive deficits within the first week of injury. Interestingly, these impairments were detected at time-points that we have previously shown single ACHI rats to no longer display such deficits, indicating that repeated ACHIs may lead to cumulative and lingering effects. No differences in locomotion or anxiety were detected between sham and ACHI rats in the first week of injury. Neuroinflammatory markers were increased in the ipsilateral hippocampus of ACHI rats when compared to sham rats at 1-week, including markers of the NLRP3 inflammasome. Analysis of additional neuropathological data, as well data from 3-month recovery rats, is ongoing.
CONCLUSION: Although preliminary, findings to date indicate that repeated ACHIs result in some neurobehavioral and neuropathological changes that appear to persist beyond that induced by a single ACHI. We hypothesise that by avoiding the potential confounds of anaesthetic and surgery, this model may be ideal for improving understanding of the effects of repeated mTBIs.

Disclosures: S.J. McDonald: None. **L. Pham:** None. **W.T. O'Brien:** None. **D.K. Wright:** None. **S.R. Shultz:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.24/G24

Topic: C.10. Brain Injury and Trauma

Support: New Jersey Commission on Brain Injury Research - CBIR17PIL007
New Jersey Commission on Brain Injury Research CBIR19IRG025
seed funds from Rowan University

Title: Time course of deficit and recovery following repetitive mild traumatic brain injury in a rodent assay of cognitive flexibility

Authors: C. KNAPP[1], B. FALLON[1], D. FOX[1], S. FLORESCO[2], R. RAGHUPATHI[3], *B. WATERHOUSE[1], R. NAVARRA[1];
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Abstract: Mild traumatic brain injury (mTBI) occurs as a result of impact to the head. mTBIs are frequently associated with sports-related activities that typically occur repeatedly over the course of an athlete's career. mTBI symptoms include impairments in prefrontal cortex (PFC) mediated functions, including attention, memory, processing speed, reaction times, and cognitive flexibility. To date, there remains a major gap in our understanding of the consequences and lasting effects of mTBI in terms of behavioral manifestations, underlying neurobiology, and potential treatment strategies; especially for repetitive mTBI events. The goal of the present work was to examine the time course of deficit and recovery from repetitive mTBI using a rodent assay of cognitive flexibility. Rats were exposed to a series of three closed head injuries (controlled cortical impact model) each separated by one week. At one, two and four weeks post final injury (PFI) they were evaluated in an automated strategy shifting task, which required the rats to learn and shift strategies according to changing task demands. Rats initially acquired a visual cue strategy in which a light illuminated above one of two levers (left or right) indicated the correct lever press response for reward. Twenty-four hours after initial acquisition, rats were assessed for retrieval of the visual cue strategy followed by a series of strategy shifting and reversal learning challenges. One week PFI, injured animals required more trials to reach criterion, omitted more trials, and performed with reduced accuracy as compared to sham controls during acquisition of the initial visual cue strategy. During the strategy shifting test, injured animals required more trials to reach criterion, omitted more trials, responded with increased latency, and performed with decreased accuracy compared to shams. Throughput scores, a performance index that blends accuracy and response speed, were also reduced in

injured animals. When evaluated again at two and four weeks PFI, all impairments observed one week PFI were no longer significantly different than sham controls. These results indicate that initial acquisition and strategy shifting performance in an operant test of cognitive flexibility are impaired but gradually recover after repetitive mTBI. Repetitive mTBI-induced deficits in cognitive flexibility can be comorbid with negative changes in arousal, attention, and decision making, all of which are mediated by PFC. As such, this model presents a useful approach for further investigating the time course of an array of behavioral deficits and potential treatment strategies following multiple mTBI insults.

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Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.25/G25

Topic: C.10. Brain Injury and Trauma

Support: NIH R01 MH101178-01
NJ Commission on Brain Injury Research CBIR17PIL007
NJ Commission on Brain Injury Research CBIR19IRG025
start-up funds from Rowan University School of Osteopathic Medicine

Title: Structural and functional attributes of the rodent central norepinephrine transmitter system following repetitive mild traumatic brain injury

Authors: *B. WATERHOUSE[1], N. JOSHI[1], D. FOX[1], R. RAGHUPATHI[2], D. DEVILBISS[1], D. CHANDLER[1], R. NAVARRA[1];
[1]Cell Biol. and Neurosci., Rowan Univ. Grad. Sch. of Biomed. Sci., Stratford, NJ; [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Repetitive concussive events (mild traumatic brain injury - mTBI) are common among high school and college athletes. Many of the cognitive deficits observed in patients after repetitive mTBI appear to be related to dysfunction of the central catecholamine transmitter systems. For example, flexible attention, the ability to engage and alternate between competing behavioral demands as reward contingencies and environmental circumstances change, is normally regulated by the dopamine and norepinephrine (NE) neurotransmission in the prefrontal cortex (PFC) and is highly susceptible to disruption by mild to moderate brain injuries. Many studies have focused on DA involvement in TBI pathology but far fewer investigations have examined the impact of TBI on NE systems. Likewise, there has been extensive study of the behavioral, physiological, and biochemical consequences of ***single*** experimentally-induced

TBI events but less is known regarding the outcomes of *repetitive* TBI in experimental animals. The goal of this exploratory study was to examine the physiology of NE-containing locus coeruleus (LC) neurons and the structural integrity and density of NE fibers in the PFC following repetitive mTBI. Rats were exposed to closed head-controlled cortical impacts (3 injuries in 1 week). Immunostaining for Fluoro Jade B (FJB), dopamine beta hydroxylase (DBH) and NE-transporter (NET) in PFC or in vitro patch clamp recording of individual LC neurons was performed six days after the final injury. FJB staining was used to confirm injury to the tissue. DBH-positive fibers (n=10 sham, n=10 injured) exhibited normal morphology but were reduced in number throughout layers III-VI of the medial and orbital PFC. NET staining was also altered in these regions. Electrophysiological measures of membrane function and cell excitability indicated hypofunction of LC neurons in injured rats (n=29 cells from 5 rats) relative to shams (n=28 cells from 3 rats). Together these results indicate reduced capacity for NE release in the forebrain including the PFC, the region of the brain responsible for multiple dimensions of executive function and the transmitter responsible for modulating PFC-mediated executive function. As such, these findings focus attention on pharmacologic manipulations of the central NE transmitter system as potential short and long-term remedies for treatment of symptoms associated with repetitive concussion.

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Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.26/G26

Topic: C.10. Brain Injury and Trauma

Support: NIH Translational Outcomes Project in Neurotrauma (TOP-NT) (UG3/UH3) grant # 1 UG3 NS106938-01

Title: MRI assessment of TBI-induced hallmark disabilities in a closed head TBI rodent model

Authors: *J. HOU[1,2], R. NELSON[1], D. PLANT[1], R. MARTIN[1], M. FEBO[3], K. K. WANG[1,4], F. J. THOMPSON[1,2,5], P. BOSE[1,2,6];
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Abstract: Traumatic brain injury (TBI) produces life-long disabilities including anxiety, balance and cognitive impairments. Our objective was to perform longitudinal (acute to chronic) *in-vivo* evaluation of TBI-induced diffuse axonal injury (DAI), microbleeds, and brain anatomical and

functional connectivity networks in function specific brain regions to better understand the TBI-induced hallmark disabilities. We used multimodal imaging strategies involving diffusion tensor mapping (DTI), BOLD-based resting state fMRI, T2* parametric mapping, and quantitative susceptibility mapping. To discern links between clinically relevant imaging markers and hallmark behavioral changes in TBI, we measured levels of anxiety-like behavior, balance and cognitive function in rats. Twenty male adult Sprague Dawley rats were randomly divided into TBI and naïve control groups. Ten rats received moderate closed head TBI (mcTBI, 450g/1.5 meter; Marmarou protocol). *In-vivo* MRI images (7T, MR Solution, UK) were taken at post-injury (p.i.) day 2 and week 4. Anxiety, balance and cognitive deficits were obtained using elevated plus maze (EPM), rotorod, and Morris water maze (MWM) at p.i. weeks 2 and 3, respectively. The EPM data revealed the TBI group spent significantly less time in the open arms (i.e. anxiety) compared to naïve group (2% vs. 22%, $p<0.001$). The TBI group also spent significantly less time on the rotorod (i.e. balance disability) than the naïve group (79.5 sec vs. 113.6 sec, $p<0.001$). Moreover, the MWM data revealed significant memory dysfunction in TBI animals. The TBI group showed significant increases in latency, and cumulative distance to find the platform compared to the naïve group (33.1 s vs. 11.7s, $p<0.05$; 21,339 cm vs. 6,322 cm, $p<0.05$). MRI images showed progressive longitudinal deterioration of DAI in functional connectivity of the central nucleus of amygdala (for anxiety function), lateral vestibular nucleus (for balance function), and hippocampus (for cognitive function). Closed head TBI induced reductions in connectivity values, node strength and organizational patterns of connectivity between nodes in the behaviorally specific regions of interest. Collectively, our study revealed significant anxiety-like behaviors, balance, and cognitive deficits in the mcTBI. These hallmark TBI-induced deficits were correlated with the significant DAI related deterioration and microbleeds in the functional connectivity networks. These preclinical MRI data revealed underlying pathological changes relating to the TBI-induced behavioral disabilities that may provide clinically relevant diagnostic and prognostic translations to human study.

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Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.27/G27

Topic: C.10. Brain Injury and Trauma

Support: Citizens United for Research in Epilepsy (T.S.)
VA Career Development Award (R.K.)
Dept. of Defense CDMRP DR080424 (T.S., P.R.)

Title: Brief treatment with 2DG reduces post-traumatic epilepsy with frequent focal and generalized seizures after CCI-induced TBI in unique "fast" kindling-susceptible PPKS rats

Authors: *T. P. SUTULA[1], R. KOTLOSKI[2], P. A. RUTECKI[2];
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Abstract: The delayed onset of human post-traumatic epilepsy (PTE) after traumatic brain injury (TBI) supports the view that PTE is a slowly evolving process of acquired epileptogenesis after initial injury, and potentially shares common mechanisms with slowly evolving processes of circuit plasticity such as lesion-induced pathway reorganization and seizure-induced kindling. We previously reported that a unique strain of "fast" perforant path kindling-susceptible (PPKS) rats (Langberg et al., Neurobiol Dis 85:122, 2015) has a high incidence (~53%) of frequent post-traumatic focal and generalized spike-wave seizures (focal+GSWS) accompanied by motor "freezing" with durations of > 3 to as long as 20-30 seconds (Rutecki et al., Soc Neurosci Abstr, 2017). The focal+GSWS are not observed in a companion "slow" perforant path kindling-resistant (PPKR) strain or outbred Sprague Dawley rats, occur as often as several times per hour as soon as 1 month after CCI, and progress in frequency and duration for as long as 12 months. 2-deoxy-D-glucose (2DG) is a glycolytic inhibitor with novel acute anticonvulsant actions and chronic antiepileptic "disease-modifying" effects indicated by an ~ 2-fold reduction in kindling progression (Stafstrom et al., Ann Neurol 65:435, 2009). Administration of 2DG by gavage immediately after CCI at a dose of 250 mg/kg/day for 2 weeks reduced the incidence of PTE manifesting by frequent prolonged focal+GSWS as well as Class V seizures in PPKS rats compared to saline-treated controls by > 50% (53% vs. 20%, $p < 0.035$, chi-square). The effect of 2DG was dose-dependent, as treatment with 50 mg/kg/day demonstrated frequent focal+GSWS as in saline-treated controls. To further evaluate the time course of action of the disease-modifying and antiepileptogenic effects of 2DG against development of PTE, we examined effects of 2DG (250 mg/kg/day) administered by gavage for 1 or 3 days after initial CCI. Frequent prolonged focal+GSWS as well as Class V seizures were observed in only 8 of 24 rats (33%) treated for 3 days and in 10 of 25 rats (40%) treated for only 1 day compared to matched saline-treated controls demonstrating focal+GSWS and Class V seizures in 29 of 48 rats (~60%). The proportions of animals demonstrating seizures in the 14 day (20%), 3 day (33%), and 1 day (40%) treatment groups are significantly different compared to saline controls (60%) (Chi-square = 10.583 with 3 degrees of freedom, $p = 0.014$). The results demonstrate that brief treatment with 2DG (250 mg/kg/day) for periods as short as 1-14 days significantly reduces PTE after CCI in rats with genetic background conferring susceptibility to seizure-induced circuit plasticity.

Disclosures: **T.P. Sutula:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); T.S holds intellectual property related to 2DG.. **R. Kotloski:** None. **P.A. Rutecki:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); P.A.R. holds intellectual property related to 2DG..

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.28/G28

Topic: C.10. Brain Injury and Trauma

Support: VA RR&D Grant I01RX001520
VA BLR&D Grant I21BX003815
DOD CDMRP Grant W81XWH-16-1-0626
The Veterans Bio-Medical Research Institute

Title: Simple weight-drop model of closed head diffuse traumatic brain injury in rats without preparatory surgery

Authors: *V. DELIC[1], J. A. BURTON[1], K. J. STALNAKER[1], K. C. PANG[1,2], K. D. BECK[1,2], B. A. CITRON[1,2];
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Abstract: Traumatic brain injury (TBI) is associated with an increased risk of developing chronic traumatic encephalopathy (CTE) and Parkinson's disease (PD) that together impact millions of people. Whether TBI causes these diseases or accelerates their underlying pathology remains unknown. Increased focus on the role of inflammation in pathogenesis of neurodegenerative diseases and extensive use of rats in studying PD necessitated the development of a rat model of TBI that does not involve preparatory surgeries which themselves can elicit a strong immune system responses. Moreover, repetitive TBIs, which are associated with the highest risk for neurodegenerative diseases, have not received sufficient attention in rats. While closed head weight drop models have been used for decades in mice, they have not been in widespread use in rats. We therefore created a simple weight drop model, building on our previous work and models developed by Buchele, Marmarou, and Pick, that is able to cause closed head TBI that is also amenable to repetitive blunt trauma to the skull. Using stainless steel rods dropped from a distance of 25 cm, we are able to achieve a repetitive TBIs. The rats are placed on a sponge that allows rapid rotational acceleration in addition to the impact injury. Injury titration was performed with 0.0 to 2.0 kg rods. A 1.75 kg rod, with a calculated 4.3 J impact, provided the highest survivable injury (n=5). Four repetitive injuries, using a 1.0 kg rod did not produce motor deficits as determined by the open field test when the traumas were separated by 10 days. Closer titrations of the impacts will provide an optimal model. Initial hematoxylin and eosin staining revealed that a repetitive 1 kg injury resulted in no overt pathology. Further histological staining will determine the degree of neurodegeneration, presence

of protein aggregates associated with neurodegenerative diseases (e.g., CTE, PD), increased inflammation, and infiltration of the peripheral phagocytic cells to sites of ongoing pathology.

Disclosures: V. Delic: None. **J.A. Burton:** None. **K.J. Stalnaker:** None. **K.C. Pang:** None. **K.D. Beck:** None. **B.A. Citron:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.29/G29

Topic: C.10. Brain Injury and Trauma

Support: VA CDA-2 (IK2BX002986)

Title: Influence of genetic background and anesthesia duration on hippocampal BDNF after acute TBI in rat

Authors: D. A. CORBIER DE LARA[1], S. DUNN[1], T. P. SUTULA[2], *R. J. KOTLOSKI[2]; [1]Univ. of Wisconsin-Madison, Madison, WI; [2]Neurol., Univ. of Wisconsin Sch. of Med. and Pub, Madison, WI

Abstract: TBI and its residuals are major health problems, with very limited treatments at present. Genetics are known to influence outcome following TBI. Prior research utilizing a pair of unique and complementary inbred rat strains selected for either increased or decreased rates of perforant path (PP) kindling, PP kindling susceptible (PPKS) and PP kindling resistant (PPKR), has demonstrated significant differences in sequelae of TBI. This study examined the effects of genetic background on gene expression and protein levels of BDNF, as well as in the influence of duration of anesthesia. Controlled cortical impact (CCI) under isoflurane was induced over the right cortex to produce a moderate-to-severe TBI. One group of PPKS (n=6) and PPKR (n=6) rats were maintained under isoflurane (2% in O_2) for 15 min after the CCI, while a second group of PPKS (n=5) and PPKR (n=6) rats were treated identically, except that they were maintained under isoflurane (2%) for 60 min following the CCI. Rats were allowed to recover and then were euthanized at 4 hrs. Controls from the PPKS (n=6) and PPKR (n=6) strains were included. The hippocampus ipsilateral to the injury was dissected and homogenized. BDNF protein levels were determined by ELISA. Data was analyzed by a standard least squares model. Results in pg/mg total protein are presented as mean±SEM. PPKS and PPKR strains demonstrated increases in hippocampal BDNF as compared to baseline following CCI and 15 min of isoflurane anesthesia (PPKS baseline 175.4±5.7, 15 min isoflurane 252±14.7; PPKR baseline 98.0±12.8, 15 min isoflurane 242.4±19.2). Comparison of the hippocampal BDNF after 15 or 60 min of isoflurane across the PPKS and PPKR strains demonstrated a significant interaction, with PPKS rats demonstrating a decrease in BDNF with 60 min of isoflurane (188±20.5) as compared to 15 min

of isoflurane (252±14.7), while PPKR rats did not demonstrate significant change with 60 min of isoflurane (235.7±16.0) as compared to 15 min of isoflurane (242.4±19.2). BDNF in the PPKS rats with 60 min of isoflurane were not different than PPKS controls (188±20.5 vs 175.4±5.7). The strain-specific differences described demonstrate that selective genetic pressures to increase or decrease the rate of PP kindling also influence hippocampal BDNF in response to acute TBI, specifically a significant interaction of strain and duration of isoflurane anesthesia duration. The findings presented demonstrate a potential mechanism of action, dependent on genetic background, for the use of induced coma in acute TBI. As BDNF is known to have important roles in epileptogenesis, these differences may presage differences in sequelae of TBI such as post-traumatic epilepsy.

Disclosures: D.A. Corbier de Lara: None. **S. Dunn:** None. **T.P. Sutula:** None. **R.J. Kotloski:** None.

Poster

050. Brain Injury and Trauma I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 050.30/G30

Topic: C.10. Brain Injury and Trauma

Title: Uninterrupted *in vivo* cerebral microdialysis measures of the acute neurochemical response to a concussion in a rat model combining force and rotation

Authors: *I. O. MASSE[1], L. MOQUIN[2], C. PROVOST[1], S. GUAY[1], A. GRATTON[2], L. DE BEAUMONT[1];
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Abstract: *Object:* Concussions/mild traumatic brain injury (mTBI) represent a major public health concern due to persistent behavioral and neurological effects. The mechanisms by which concussions lead to such effects are partly attributable to neurotransmitter and synaptic alterations. For example, cerebral microdialysis studies in rodents reported a peak of extracellular glutamate 10 minutes after injury. Microdialysis has the advantage of being one of the few techniques allowing the quantification of neurotransmitters *in vivo* and at different time points following injury. In addition to the clear advantages afforded by microdialysis, the Wayne State weight-drop model induces an impact on the skull of a subject unrestrained by the fall of a weight. The latter model exerts rapid acceleration and deceleration of the head and torso, an essential feature in human craniocerebral trauma and a factor that is missing from many existing animal concussion models. *Methods:* In the present study, we applied the Wayne State procedure and microdialysis to document, in awake rats, the acute changes in extracellular glutamate, glycine, GABA and taurine levels resulting from concussive trauma. The microdialysis probe

was inserted inside the CA1 region of the hippocampus as region of interest and was left inserted in the brain at impact. The hippocampus contains a high density of terminals and receptors, thus making it a relevant region to study alterations in amino acids levels following concussion. Using HPLC, dialysate levels of hippocampal glutamate, glycine, GABA and taurine were measured in adult male Sprague-Dawley rats in 10 min increments for 60 min prior to, during and for 90 min following concussive trauma induced by the Wayne State weight-drop procedure. Sham control animals were treated in the same manner but without receiving the concussive trauma procedure. *Results:* Our results show that concussive trauma is followed, within 10 min, by a robust, transient 4-fold increase in hippocampal glutamate and taurine levels, and a 2-fold increase in glycine levels; such changes were not seen in controls. In contrast, hippocampal GABA levels were not significantly affected by the concussive trauma procedure. *Conclusions:* The findings derived from the approach used here are generally consistent with previously demonstrated post-concussion symptomology. They also provide a crucial *in vivo* validation of the Wayne State procedure as a model with promising translational potential for pre-clinical studies on early therapeutic responses to concussion.

Disclosures: I.O. Masse: None. **L. Moquin:** None. **C. Provost:** None. **S. Guay:** None. **A. Gratton:** None. **L. De Beaumont:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.01/G31

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NJCSCR Grant CSCR17IRG007
The Reynolds Family Spine Laboratory Funds

Title: Toll-like receptor 9 antagonism preserves proximal corticospinal tract axons following spinal cord injury

Authors: *L. LI, L. NI, R. F. HEARY, S. ELKABES;
Neurolog. Surgery, NJMS, Rutgers Univ., Newark, NJ

Abstract: Innate immune receptors play important roles in traumatic central nervous system injury. Despite advances, the contribution of toll-like receptors (TLRs) to spinal cord injury (SCI) remain inadequately defined. We previously reported that a TLR9 antagonist, oligodeoxynucleotide 2088 (ODN 2088), administered intrathecally, significantly decreases chondroitin sulfate proteoglycan (CSPG) immunoreactivity and astrocyte proliferation at the glial scar in mice sustaining a severe mid-thoracic (T8) SC contusion injury as compared to injured controls treated with vehicle (Li et al, 2019). Using the same animal model, the present

studies investigated whether ODN 2088 modulates microglia/macrophage proliferation at the glial scar and how the overall changes at the injury site affect the preservation and re-growth of injured axons.Mice sustaining a SCI received EdU, a thymidine analog which incorporates into the DNA of proliferating cells. Microglia/macrophages were visualized by immunohistochemistry using an antibody against Iba-1. Stereological approaches were utilized to quantify EdU^+/Iba-1^+cells in regions immediately adjacent to the lesion border. There was a 54% decrease in the number of EdU^+/Iba-1^+cells in ODN 2088-treated injured mice compared to vehicle-treated injured controls. These results indicated that ODN 2088 attenuates the proliferation of microglia/macrophages at the glial scar.To determine the effects of ODN 2088 on injured CST axons, we injected the anterograde axonal tracer biotinylated dextran amine (BDA) into the somatosensory cortex and quantified the number of labeled axons in regions caudal and rostral to the lesion border. The number of BDA^+axons was 8-fold, 4-fold and 2-fold higher in ODN 2088-treated injured mice compared to vehicle-treated injured mice, at 180, 360 and 540 μm distance to the rostral lesion border, respectively. In contrast, we did not observe BDA^+axons beyond the glial scar in ODN 2088- or vehicle-treated injured mice. These results suggest that ODN 2088 preserves proximal CST axons but does not foster axonal regeneration beyond the glial scar. Accordingly, evaluation of open field locomotor activity using the Basso Mouse Scale (BMS) sub-scores, which can detect subtle differences in motor function and coordination, did not show motor improvement following ODN 2088 treatment. This result is consistent with the lack of CST axon re-growth beyond the glial scar. Ongoing studies are determining whether ODN 2088 prevents axonal dieback or promotes the growth of proximal axons after they retract from the lesion site. The possibility of increased collateral sprouting in proximal axons is also investigated.

Disclosures: **L. Li:** None. **L. Ni:** None. **R.F. Heary:** None. **S. Elkabes:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.02/G32

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Shriners Hospitals for Children # 85120

Title: Axon injury induces fragmentation of axonal mitochondria

Authors: *J. KEDRA, S. LIN, G. GALLO, G. SMITH;
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Abstract: Unlike axons of the peripheral nervous system (PNS), axons of the central nervous system (CNS) demonstrate poor regenerative ability, even with enhanced intrinsic or extrinsic

environments. We sought to investigate the role of mitochondria in the axonal response to injury. A viral vector (AAV) containing a mitochondrially targeted fluorescent protein (mitoDsRed) as well as fluorescently tagged LC3 (GFP-LC3), an autophagosomal marker, was injected into either primary motor cortex, to label the corticospinal tract (CST), or into the sciatic nerve. The axons of the CST and sciatic nerve were then injured by either dorsal hemisection at C5 or sciatic nerve transection. We found that mitochondria in injured CST axons near the injury site are significantly smaller compared to uninjured controls and the reduced size persists for two weeks before returning to pre-injury levels, while mitochondria in injured PNS axons also show reduced size, but to a lesser degree. Additionally, it was determined that there is increased mitophagy in CST axons following spinal cord injury based on increased colocalization of mitochondria and LC3. To investigate the molecular mechanisms regulating the decreased mitochondrial length we employed an *in vitro* embryonic chicken DRG model. Live imaging of fluorescently labeled mitochondria *in vitro* after axotomy directly revealed the fragmentation of mitochondria underlying the decrease in length. To address whether the observed decrease in mitochondria length following axon injury is reflective of fission both *in vitro* and *in vivo* studies utilized a specific pharmacological inhibitor of Drp1 (mDivi-1), the GTPase responsible for mitochondrial fission. mDivi-1 inhibited the axotomy-induced fragmentation of mitochondria both *in vitro* and *in vivo*. Additionally, the role of mitochondrial calcium uptake in injury induced fission was examined *in vitro*. It was found that pharmacologically blocking mitochondrial calcium uptake using RU360, a mitochondrial calcium uniporter inhibitor, prevented injury induce mitochondrial fission. In conclusion, these studies indicate that following injury, both *in vivo* and *in vitro*, axonal mitochondria undergo increased fission, which may result in an ATP deficit that contributes to the lack of regeneration seen in CNS neurons.

Disclosures: **J. Kedra:** None. **S. Lin:** None. **G. Gallo:** None. **G. Smith:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.03/G33

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Effect of PTEN antagonist peptide on the functional motor recovery in rat

Authors: *S. LV[1], W. WU[2];
[1]Guangdong-hongkong-Macau Inst. of CNS Regeneration, Guangzhou, China; [2]The Univ. Of Hong Kong, Hong Kong SAR, Hong Kong

Abstract: Ventral root injury results in great loss of motor functions because of the inefficient axon regeneration and severe atrophy of target organ. PTEN act as a negative regulatory factor at PI3K/AKT pathway also inhibit the regeneration of axons. It has been shown that PTEN

antagonist peptides(PAPs) can significantly stimulated growth of descending serotonergic fibers and sprouting of corticospinal fibers in the rostral spinal cord after spinal cord injury. Here, we are reporting that after a spinal ventral root crush completely in adult rats, PAPs peptides treatment remarkably improved motor functional recovery. PAPs-treated animals showed less motoneuron death, increased the number of regenerated axons, rebuilt healthy neuromuscular junction and enhanced potentiated electrical responses of motor units. Our study showed that PAPs was a promising pharmacological method for promoting motor functional recovery after peripheral nerve injury.

Disclosures: S. Lv: None. **W. Wu:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.04/G34

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS101298
Craig H Neilsen Foundation 460461
State of Florida
Department of Veterans Affairs

Title: Inflammatory priming of mesenchymal stem cells improves angiogenesis in the spinal cord after contusion injury

Authors: *I. MALDONADO-LASUNCION[1,2], J. VERHAAGEN[3], M. OUDEGA[4,5];
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Abstract: Mesenchymal stem cells (MSCs) transplanted in the injured spinal cord exert paracrine actions resulting in angiogenesis, neural survival, and axon growth and remyelination, which are often accompanied by functional improvements. An intraspinal transplant of MSC encounters an active inflammatory microenvironment consisting mainly of inflammatory macrophages. MSC react to stressors by enhancing their paracrine activities. Inflammatory signals enhance growth factor secretion by MSC. We studied the effect of macrophage-mediated priming on MSC *in vitro* and *in vivo*. We exposed MSC to *in vitro*-polarized inflammatory macrophages or their conditioned medium and investigated the effects on MSC secretome and transcriptome. Exposure of MSC to macrophage-mediated inflammation increases the gene expression and secretion of vascular endothelial growth factor, which is important for the

initiation of angiogenesis, and anti-inflammatory molecules, including prostaglandin E2, indoleamine 2,3-dioxygenase, and interleukin 4, which may induce the switch in macrophage polarization to the anti-inflammatory phenotypes. Inflammatory priming of MSC also induced expression of genes related to axon regeneration and remyelination, such as glial derived neurotrophic factor and nerve growth factor. Based on these exciting *in vitro* data, we tested the effects of inflammatory-primed MSC on spinal cord repair using an adult rat model of contusive spinal cord injury. Inflammation-activated MSC induced a significant increase in the number of blood vessels in the contused spinal cord compared to non-activated MSC or vehicle, which correlated positively with spared tissue volume and negatively with the inflammatory environment. The effects of our approach on functional performance will be presented. Our results highlight the significance of macrophage-MSC interactions for MSC-mediated spinal cord repair.

Disclosures: I. Maldonado-Lasuncion: None. **J. Verhaagen:** None. **M. Oudega:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.05/G35

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Id2 promotes axonal growth through upregulation of Neurogenin2

Authors: *J. LIU[1], Z. HUANG[1], J. JIN[1], Q. CHEN[1], L. ZHOU[1], B. ZHOU[2], A. LI[1], P. YU[1]; [1]GHMICR, Jinan Univ., Guangzhou, China; [2]Advanced Innovation Ctr. for Big Data-Based Precision Med., Beihang Univ., Beijing, China

Abstract: Manipulation of developmentally regulated genes controlling neuronal differentiation and axonal growth presents a promising strategy to enhance the intrinsic growth capability of adult neurons. Inhibitor of DNA binding 2 (Id2), a negative regulator of bHLH transcriptional factors, promotes axonal growth after its forced expression in post-mitotic neurons. In this study, we investigated the mechanism of Id2 on promoting axonal growth and revealed that Neurogenin 2 (Ngn2) contributed to the growth-activating role of Id2 in neurons. Ngn2 is a neural specific bHLH factor which specifies neuronal fate and drives neuronal differentiation during development. Using RNAseq screening followed by further confirmation from its protein level both *in vitro* and *in vivo*, we found Ngn2 expression was up-regulated by Id2. Overexpression of Ngn2 in cortical neurons promoted axonal growth, and more importantly, knockdown of Ngn2, impaired the axonal growth promoting effect of Id2. Our results suggest that elevation of Ngn2 in neurons may be a potential strategy to stimulate axonal regeneration after injury.

Disclosures: **J. Liu:** None. **Z. Huang:** None. **J. Jin:** None. **Q. Chen:** None. **L. Zhou:** None. **B. Zhou:** None. **A. Li:** None. **P. Yu:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.06/G36

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NYSCIRB #DOH01-ISSCI6-2016-00018
US DOD #W81XWH-15-1-0614

Title: Longitudinal profiling of systemic T cells in persons with traumatic spinal cord injury

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[3]Physical Med. and Rehabil., Zucker Sch. of Med. at Hofstra Northwell, Great Neck, NY

Abstract: Life expectancy for persons with traumatic spinal cord injury (SCI) has not improved in decades and is lower than for able-bodied persons. Infections are the leading cause of death after SCI and are associated with poor neurological recovery. Systemic inflammation is also commonly measured in persons with SCI. The cellular mechanisms contributing to infection susceptibility and inflammation after SCI are unknown. T cells are critical for mediating the adaptive immune response. CD4+ T cells have numerous effector functions, e.g. activating other immune cells including B cells and cytotoxic CD8+ T cells, triggering antibody production, and limiting immune responses to self-antigens. Here we profiled circulating T cells from persons with SCI (N=8 total, 7 males) acutely (0-5 days post injury, dpi) and at 3, 6- and 12-months post injury (mpi). Participants had cervical and thoracic level injuries (N=4, 4), that were neurologically complete or incomplete (N=2, 6 respectively) and ranged in age (24-83 years old). Data from persons with SCI was compared to data from able-bodied (AB) persons (N=9 total, 5 males) who ranged in age 26-48 years old. Compared to AB, the percentage of T cells (CD3+) was significantly lower in persons with SCI acutely (24% vs. 47%, P=0.012), while activated CD3+CD4+ T cells, indicated by elevated HLA-DR, were increased at 3, 6 and 12mpi (P=0.01, 0.0091, 0.036 respectively). There was also an increased percentage of regulatory T cells (CD3+CD4+CD25+CD127lo) that expressed CCR4+ in persons with SCI acutely (P=0.003) and of activated regulatory T cells (CD3+CD4+CD25+CD127lo CCR4+) indicated by elevated HLA-DR at 6mpi (P=0.036). These data confirm and extend results of our previous study (Monahan et al 2015) demonstrating changes in T cell populations in persons with chronic (>12mpi) SCI. In the future, we will determine if changes in T cell subsets after SCI correlate with specific clinical or demographic characteristics or with the trajectory of neurological

recovery. These data are part of a larger ongoing study to determine biomarkers of spontaneous recovery and immunological changes after SCI.

Disclosures: C. Pinpin: None. **M.A. Bank:** None. **A.B. Stein:** None. **O. Bloom:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.07/G37

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Elucidating the neural plasticity underlying functional recovery after spinal cord injury in primates

Authors: *Y. TAKATA[1], H. NAKAGAWA[2], H. YAMANAKA[1], M. TAKADA[1];
[1]Primate Res. Institute, Kyoto Univ., Inuyama, Aichi, Japan; [2]Immunol. Frontier Res. Center, Osaka Univ., Suita,Osaka, Japan

Abstract: Manual dexterity in primates strongly correlates with the development of the corticomotoneuronal pathway. After spinal cord injury (SCI) at the cervical level, manual dexterity is severely impaired and daily activities become difficult. Previous studies have indicated that sprouting of corticospinal tract fibers after SCI is important to enhance the recovery of manual dexterity in a primate model of SCI. Furthermore, another study reported that cortical motor-related areas were activated in parallel with restoration of manual dexterity after SCI. These findings imply that the reorganization of the motor-related areas may be crucial in the recovery process of manual dexterity. However, it remains unclear how layer-V pyramidal neurons giving rise to the corticomotoneuronal pathway exhibit plasticity after SCI. Here, we examined the post-SCI morphological changes in layer-V pyramidal neurons in the motor-related areas, including the primary motor cortex (MI), the supplementary motor area (SMA), and the dorsal and ventral divisions of the premotor cortex (PMd, PMv). We compared the layer-V pyramidal neurons in normal controls and acute stage/early recovery stage SCI models. The digit region of each motor-related area was identified by intracortical microstimulation. The layer-V pyramidal neurons in individual digit regions were visualized by Golgi staining. The assessment of manual dexterity was performed by using a reaching/grasping task. For preparing SCI models, unilateral lesions were made at the C6/C7 border of the spinal cord. Acute stage models were sacrificed ten days after SCI, while an early recovery stage model was sacrificed 30 days post-SCI. At the moment of sacrifice, the former models still exhibited severe impairments on the injured side, and manual dexterity of the latter model was spontaneously restored. In our analyses, we found that throughout the motor-related areas, the dendritic complexity of the pyramidal neurons was reduced and, also, their dendritic spine density was decreased in the acute stage models as compared to the normal controls. By contrast, no significant difference in the

dendritic complexity of the pyramidal neurons in MI, PMd, and PMv was observed between the early recovery and the control group, and no significant difference in the dendritic spine density in SMA and PMd was seen between two groups.

Disclosures: **Y. Takata:** None. **H. Nakagawa:** None. **H. Yamanaka:** None. **M. Takada:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.08/G38

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Natural Science Foundation of China Grants: 81671096
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Jiangsu Project Funded by the Qing Lan Project,
National Natural Science Foundation of China Grants: 81701107

Title: circRNA-Kat6b overexpression attenuated nerve injury-induced nociceptive hypersensitivity via rescuing Kcnk1 in spinal cord neurons

Authors: L. XIE, Q. ZHANG, X. LIU, H.-M. ZHOU, L.-Y. HAO, ***Z. PAN**;
Xuzhou Med. Univ., Xuzhou, China

Abstract: Dysfunctions of gene transcription and translation in the nociceptive pathways play the critical role in the progress of neuropathic pain. Circular RNAs (circRNAs) are emerging as new players in regulation of gene expression, however, little is known about whether and how circRNAs are involved in neuropathic pain. In this present study, we found that chronic constriction injury (CCI)-induced neuropathic pain significantly decreased circRNA-Kat6b expression in spinal neurons of mice. Overexpression of circRNA-Kat6b alleviated the pain sensitivity to the thermal and mechanical stimulus, and knockdown of circRNA-Kat6b mimicked the nociceptive behaviors as evidenced by decreased thermal and mechanical nociceptive threshold. Furthermore, miRNA-26a was increased in the spinal cord of CCI mice, and was validated to be a binding target of circRNA-Kat6b. Knockdown of spinal miRNA-26a attenuated the pain, overexpressing miRNA-26a induced the produce of pain sensitivity. Finally, Kcnk1 was predicted to a target of miRNA-26a, overexpression of miRNA-26a inhibited the activity of luciferase reporter. Contrarily, downregulation of miRNA-26a increased the reporter activity. Increase of Kcnk1 expression inhibited the pain-like behavior induced by the decreased circRNA-Kat6b or increased miRNA-26a in naïve mice. Collectively, we demonstrated that

circRNA-Kat6b regulated neuropathic pain via targeting miRNA-26a/Kcnk1 at the spinal cord level. This study will reveal a new functional regulatory molecular mechanism underlying chronic inflammatory pain process, which will provide a possible target for the development of analgesic drugs.

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Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.09/G39

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life Spinal Cord Injury Foundation
Department of Neurosurgery, MCW

Title: Role of macrophage inflammatory protein-1alpha (CCL3) in secondary damage following contusive spinal cord injury

Authors: *N. PELISCH[1,2], K. STEHLIK[1,2], B. APERI[1,2], A. KRONER[1,2];
[1]Med. Col. of Wisconsin, Milwaukee, WI; [2]Clement J. Zablocki VA Med. Ctr., Milwaukee, WI

Abstract: Preserving white matter tissue following spinal cord injury (SCI) is an important therapeutic goal to minimize tissue damage and improve neurological outcome; however, the complex cellular and molecular mechanisms that mediate secondary damage are not fully understood. Secondary damage following SCI occurs due to a sequence of events after the primary injury, including hemorrhage and inflammation, contributing to increased lesion size and poor locomotor recovery. We have assessed the expression of CCL3, a member of the CC chemokine family, and its receptors at different timepoints after contusive SCI. The expression levels of CCL3 and its receptors CCR1, CCR4 and CCR5 were characterized at the lesion site of female 6-8 week old C57BL/6 mice with a moderate T11 contusion injury. CCL3 was upregulated after injury, with a peak at 6 hours and stayed upregulated for the duration of the experiment (28 days). Similarly, CCR1 and CCR5 expression was also increased at day 3 and 7-post injury, respectively. CCR4 in contrast did not show any significant change ($p < 0.05$, $n = 4$-5). Next, we compared locomotor recovery in CCL3 knockout and wild type mice. The Basso Mouse Scale locomotor score (BMS) showed that the CCL3 knockout mice initially recovered better compared to the wild type group, but at later time points, the scores of the CCL3 knockout mice started to decline, while the wild type group showed a slight improvement ($p < 0.05$, $n = 9$-10), which could be indicative of a biphasic effect. To address the role of CCL3 in mediating secondary damage therapeutically, we knocked-down CCL3 using a novel technology of

antisense oligonucleotides. FANA antisense oligonucleotides (FANA ASO) can penetrate cells and tissues without the need of transfection. We used adult female C57BL/6J mice and intrathecally delivered FANA ASO to specifically knock down CCL3 or a scrambled control (10mg/kg) immediately after contusion injury. We report that knocking down CCL3 after SCI significantly decreased the expression of CCL3 and its receptor CCR5 at day 3-post contusion ($p < 0.05$, $n = 5$-6). In summary, we suggest that the proinflammatory chemokine CCL3 might be critically involved in the pathophysiology of inflammation and contribute to secondary damage following contusive SCI *in vivo*, thereby providing a new potential target for SCI therapy.

Disclosures: **N. Pelisch:** None. **K. Stehlik:** None. **B. Aperi:** None. **A. Kroner:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.10/G40

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Shriners Hospital for Children 86700-NCA-17
NSF 1754340
NIH R01NS073055

Title: Calcium and ERK1/2 signaling during spinal cord and skeletal muscle regeneration in the tail of *Xenopus laevis* larvae

Authors: *J. B. LEVIN[1], L. N. BORODINSKY[2];
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Abstract: Great strides have been made in identifying processes that keep stem cells competent during periods of inactivity, and support function after activation; however, the integration of these pathways to decide stem cell action is still unclear. Ca^{2+} and the extracellular signal-regulated kinase (ERK1/2) are two signaling pathways that are sensitive to many other signals and have the potential to coordinate them. We hypothesize that a precise spatial and temporal regulation of both of these signals in regenerating tissues determines their effect. Both Ca^{2+} and ERK1/2 play important roles during early development but their functions during regeneration are not fully deciphered. To investigate this, our project utilizes a model organism that is proficient at regeneration to show that both Ca^{2+} and ERK1/2 signaling pathways are important for regeneration. Utilizing a genetically-encoded Ca^{2+} indicator (GCaMP6s) expressed in stage-39 *Xenopus laevis* larvae, we show *in vivo* that Ca^{2+} signaling is stimulated after injury in multiple tissues, including spinal cord, and persists during regeneration. Immunohistochemistry shows that ERK1/2 signaling is also activated in regenerating muscle and neural stem cells.

Using an inhibitor of MEK1/2 function, we define interplay between Ca^{2+} and ERK1/2 where ERK1/2 action is necessary for an injury-induced increase in Ca^{2+} activity in tissues of the tail but not in the spinal cord. In addition, we show that ERK1/2 activation is necessary for effective regeneration of both spinal cord and muscle, and influences proliferation of regenerating tissues. However, enhanced activation of ERK1/2 signaling using an optogenetic approach does not enhance regeneration, demonstrating that the temporal regulation of ERK1/2 signaling determines its effectiveness. Finally, using simultaneous live-imaging of Ca^{2+} and ERK1/2 dynamics, we discover that Ca^{2+} transients inhibit ERK1/2 signaling in cultured embryonic neurons but not embryonic muscle cells, suggesting that the interaction between Ca^{2+} and ERK1/2 is distinct between these cell types. Altogether, these results suggest that ERK1/2 and Ca^{2+} signaling interact in a manner that modifies their temporal activation, which is important to promote successful regeneration.

Disclosures: **J.B. Levin:** None. **L.N. Borodinsky:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.11/G41

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Science Centre Preludium Grant UMO-2016/23/N/NZ4/03337
NSC OPUS Grant UMO-2013/09/B/NZ4/03306

Title: Time-dependent changes in the perineuronal nets of transected spinal cord: Differential responses in thoracic regions of scar formation and in extracellular milieu of lumbar motoneurons

Authors: *M. H. SKUP, **K. GRYCZ**, O. GAJEWSKA-WOZNIAK, J. CZARKOWSKA-BAUCH;
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Abstract: The perineuronal nets (PNNs) in the mature CNS maintain the architecture and stabilize synapses, thereby reducing synaptic plasticity. In the ventral spinal cord PNNs encapsulate predominantly α-motoneurons (MNs), suggesting significance of PNNs in control of MNs inputs and activity. We have shown previously both spontaneous and exercise-stimulated reorganization of inputs to lumbar MNs at the 2^{nd} and 6^{th} week after complete transection of the rat spinal cord (SCT) (Skup et al., 2012, Gajewska-Wozniak et al., 2016). Changes in the inputs raised the question whether expression patterns of essential components of PNNs, chondroitin sulfate proteoglycans (CSPGs), change in parallel. To answer that question the CSPGs gene transcripts and protein levels were examined at 2 and 5 weeks after SCT in control (n=5) and

spinal (n=6-8/group) rats. To quantify gene expression qRT-PCR was carried out and expression levels were related to internal control genes (GAPDH, Actin) as the $C_{T.}$ The protein level was quantified by ELISA. SCT led to an early, five-fold decrease of Crtl1/Hapln1 link protein and to concomitant, four-fold increase of neurocan transcripts at the lesion site, progressing in time; the latter was accompanied by two-fold increase of protein level. A 30% decrease of Crtl1/Hapln1 link protein reached the lumbar segments in the chronic postlesion period. In search for the mechanisms which underlie selective decrease of cholinergic innervation of MNs controlling the ankle extensor (soleus) but not flexor muscles (tibialis anterior) which were shown 6 weeks after SCT (Skup et al., 2012), we assumed that PNNs limit network reorganization after SCT and hypothesized, that CSPGs deposition is regulated differentially around extensor and flexor MNs. Using immunofluorescence to identify CSPGs and imaging of tracer-identified MNs we found that early after SCT the level of Crtl1/Hapln1 did not change as compared to control, but at 6 weeks it decreased by 30% around extensor but not flexor α-MNs. In conclusion, the SCT-caused spatiotemporal changes of CSPGs gene expression, accompanied by less changes in CSPGs proteins, indicate early and progressing destabilization and reorganization of the PNNs. The mechanism downregulating Crtl1/Hapln favouring permissive milieu around the extensor MNs at the chronic post-lesion stage, might enable compensation of their synaptic deficit.

Disclosures: M.H. Skup: None. **K. Grycz:** None. **O. Gajewska-Wozniak:** None. **J. Czarkowska-Bauch:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.12/G42

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NJCSCR grant CSCR12IRG007
NJCSCR grant CSCR17IRG007
The Reynolds Family Spine Laboratory Funds

Title: Differential effects of toll-like receptor 2 stimulating ligands on the viability of spinal cord neurons in an injury model *in vitro*

Authors: *C. ACIOGLU, R. F. HEARY, S. ELKABES;
The Reynolds Family Spine Laboratory, Dept. of Neurolog. Surgery, New Jersey Med. School, Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Toll-like receptor 2 (TLR2) is a pattern recognition receptor that initiates an inflammatory reaction in response to danger signals that are released by damaged or dying cells following tissue injury. Studies have shown that genetic deletion of TLR2 exacerbates the

outcomes of spinal cord injury (SCI) whereas stimulation of TLR2 signaling exerts protective effects in SCI. There is evidence that some of these protective effects are mediated by glia and especially, microglia and macrophages. However, we found that SC neurons express TLR2. It is not yet known whether direct stimulation of neuronal TLR2 protects neurons against injury. To address this issue, we established pure SC neuronal cultures utilizing C57Bl/6 mouse embryos and maintained them 10 days *in vitro* to allow maturation. Kainic acid (KA; 20 μM) was used to induce an excitotoxic insult in the presence or absence of zymosan (5 μg/ml), an agonist that is known to stimulate the TLR2/TLR6 complex or Pam3CSK4 (200 ng/ml) an agonist that primarily activates the TLR2/TLR1 complex. Neuronal survival was assessed by counting the β-tubulin III immunoreactive cells after treatment with vehicle, KA, and/or TLR2 agonists for 24 hours. Combined data from three independent experiments with 4-5 sister cultures/group indicated that KA induces a significant 33.6% $\pm$4.2 decrease in neuron number compared to vehicle-treated cultures ($p<0.0001$ by ANOVA). Addition of zymosan or Pam3CSK4 to the cultures neither protected against KA-induced death nor exacerbated excitotoxic neuronal loss. Surprisingly, there was a significant 24.3% $\pm$4.2 decrease in the number of neurons in cultures treated with zymosan alone ($p<0.0001$ by ANOVA) in the absence of KA, whereas such deleterious effects were not observed in cultures treated only with Pam3CSK4. Addition of zymosan to cultures of cortical neurons did not promote neuronal loss despite expression of TLR2 and TLR6. Based on these findings we postulate that the neuroprotective effects of TLR2 in SCI are likely mediated through TLR2 signaling in glia or infiltrating immune cells and not via direct stimulation of TLR2 in SC neurons. We further propose that TLR2 stimulation can reduce the viability of uninjured SC neurons *in vitro*, but this effect is ligand- and potentially, co-receptor dependent. The vulnerability of neurons to zymosan-induced TLR2 activation also depends on the neuronal type, with SC neurons being a susceptible neuronal population.

Disclosures: C. Acioglu: None. **R.F. Heary:** None. **S. Elkabes:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.13/G43

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NRF-2019R1C1C1005728

Title: Identification of function of synaptic cell adhesion molecule 3 as a new therapeutic target ine spianl cord injury model

Authors: *J. KYUNG[1], I. HAN[2];
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Abstract: SynCAM3 is a neural cell adhesion molecule that is expressed in CNS synapse. SynCAM3 have been shown to be implicated in synapse formation, maturation, and synaptic plasticity. Previous studies have indicated that synaptic plasticity play a critical role in spinal cord injury. However, while the understanding of mechanisms underlying synaptic plasticity in SCI has progressed, there remain several controversial points. In the present study, we explored whether after spinal cord injury SynCAM3 is involved in neural connection. SynCAM3 deficiency impaired synaptic transmission and reavailability with inducing structural alterations in synapses. After spinal cord injury, neuronal SynCAM3 promote maintenance of synapse and regulate neural growth. The defective neural growth shown in SynCAM3 KO neurons may be attributable to alterations in other adhesion molecule level. Hence, the results demonstrated that SynCAM3 appears essential for synaptic transmission and reavailability. after spinal cord injury, SynCAM3 can promote synaptic plasticity and synapse remodeling.

Disclosures: **J. Kyung:** None. **I. Han:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.14/G44

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ERA-Net NEURON grant
FRQS grant

Title: TLR4 signaling contributes to increased scar formation myelin and neuronal loss and reduced locomotor recovery after spinal cord injury

Authors: *F. RYAN[1], E. J. BRADBURY[2], S. DAVID[1];
[1]McGill Univ. Hlth. Ctr., Montreal, QC, Canada; [2]King's Col. London, London, United Kingdom

Abstract: Inflammation at the injury site contributes importantly to secondary damage and functional outcomes after spinal cord injury. Toll-like receptor (TLR) signaling in response to molecules released by damaged tissue occur very rapidly after injury. Here we studied the role of TLR4 in spinal cord injury in adult mice. To do this, spinal cord contusion injuries were performed in female TLR4 knockout and wildtype mice at 8-10 weeks of age using the Infinite Horizon impactor. Locomotor recovery was evaluated over an 8-week period using the Basso Mouse Scale (BMS) and DigiGait analyses. The BMS analysis showed a small but significant improvement in locomotor recovery in the TLR4 KO mice at the 8-week timepoint as compared to wildtype SCI controls. Analysis of the BMS sub-scores, which evaluate finer aspects of locomotor control showed significant improvement in TLR4 KO mice starting from day 35 onwards (n=10-12 mice). DigiGait analysis also showed improvement in several parameters of

gait control in TLR4 KO mice compared to WT injured controls. This included significant increase in stride length, duration of the swing phase and propulsion, and reduction in stride frequency of stepping and brake speed, indicators of improved locomotor control. Immunofluorescence staining was carried out to assess the role of TLR4 in the expression of chondroitin sulfate proteoglycans (CSPG) in the extracellular matrix using the CS-56 antibody and biotinylated Wisteria floribunda agglutinin (WFA) to identify CSPGs in the scar and perineuronal nets, respectively. These sections were double labeled with GFAP, CD11b and NeuN. Greater levels of WFA and lower levels of CS-56 staining was found in the injured spinal cord in TLR4 KO mice compared to WT mice at both 7 days and 8 weeks post-SCI. There were also greater numbers of NeuN+ neurons in the ventral grey matter in TLR4 KO mice at both survival times as compared to WT mice. Quantification of myelin staining performed using Luxol Fast Blue showed significant reduction in myelin loss in TLR4 KO mice at 8 weeks post-SCI. These results suggest that TLR4 signaling contributes to increased scar formation, increased myelin and neuronal loss, and reduced locomotor recovery after SCI.

Disclosures: F. Ryan: None. **E.J. Bradbury:** None. **S. David:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.15/H1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 1 ZIA NS003153 02
European Research Council (ERC) Consolidator (ERC-2015-CoG 682999)

Title: System-wide changes at a single cell resolution: Profiling the lumbar spinal cord following thoracic contusion

Authors: *K. MATSON[1], D. RUSS[2], C. KATHE[3], A. SATHYAMURTHY[1], J. SQUAIR[3], G. COURTINE[3], A. LEVINE[1];
[1]NINDS, [2]CIT, NIH, Bethesda, MD; [3]Swiss Federal Inst. of Technol. Lausanne, Geneve, Switzerland

Abstract: Injury to the spinal cord elicits a wide range of biological changes, ultimately disrupting neural circuitry and resulting in paralysis. The biological response to spinal cord injury (SCI) is multi-faceted, with microglia, astrocytes, oligodendrocytes and a diverse array of neurons changing their molecular programs and cell states. Therefore, effective treatments must take these complex changes into account. However, current techniques limit our understanding of such a complex system. By studying the role of individual cell types, we are unable to understand the entire system, and by studying bulk changes in gene expression we cannot

examine rare cell types or subpopulations. Recently, we adapted RNA sequencing with single cell resolution to profile genes expressed in each of the cell types in the lumbar spinal cord in an unbiased high-throughput manner. This method of single nucleus RNA sequencing (snRNA-Seq) avoids experimentally-induced gene expression and selective cell death common in single cell profiling. Using snRNA-Seq on the adult mouse lumbar spinal cord, we identified major cell types as well as 43 neuronal populations, creating an atlas for the field. We have since further optimized this protocol to yield greater throughput per sample in a robust manner, opening the door to using more precious and variable samples such as diseased or injured spinal cord. With single cell resolution and massively parallel size, this will allow us to investigate the response of all cells, from major cell types to rare populations in the lumbar spinal cord following injury. We applied snRNA-Seq to profile over 70,000 nuclei from lumbar segments of intact and thoracically contused mouse spinal cords, capturing immense complexity of the molecular changes within and between cell types. Ongoing work includes validating findings and investigating cell-type specific and between-cell molecular changes following SCI. This work enables the study of gene expression at both a cellular resolution and a system-wide perspective; thereby identifying network-level changes following SCI as well as potential targets for therapy and functional recovery in the lumbar spinal cord.

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Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.16/H2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Basic Research Program of China (973 Program, 2014CB542205)
the National Natural Science Foundation of China (81641047)

Title: Effect of VEGF on inflammatory regulation and functional improvement in rats following a complete spinal cord transection

Authors: *J. LI[1], Q. WEN[1], L. ZHOU[2], W. WU[3];
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Abstract: After complete transection of the thoracic spinal segment, neonatal rats exhibit spontaneous locomotor recovery of hindlimbs, but this recovery is not found in adult rats after similar injury. The potential mechanism related to the difference in recovery of neonatal and adult rats remains unknown. The vascular endothelial growth factor (VEGF) level in spinal

segments below injury sites was significantly higher in postnatal day 1 rats (P1) compared with 28-day-old adult rats (P28) following a complete T9 transection. VEGF administration in P28 rats with T9 transection significantly improved the functional recovery; by contrast, treatment with VEGF receptor inhibitors in P1 rats with T9 transection slowed down the spontaneous functional recovery. Results showed more neurons reduced in the lumbar spinal cord and worse local neural network reorganization below injury sites in P28 rats than those in P1 rats. Transynaptic tracing with pseudorabies virus and double immunofluorescence analysis indicated that VEGF treatment in P28 rats alleviated the reduced number of neurons and improved their network reorganization. VEGF inhibition in neonates resulted in high neuronal death rate and deteriorated network reorganization. In in vivo studies, T9 transection induced less increase in the number of microglia in the spinal cord in P1 animals than P28 animals. VEGF treatment reduced the increase in microglial cells in P28 animals. VEGF administration in cultured spinal motoneurons prevented lipopolysaccharide (LPS)-induced neuronal death and facilitated neurite growth. Western blots of the samples of lumbar spinal cord after spinal transection and cultured spinal motoneurons showed a lower level of Erk1/2 phosphorylation after the injury or LPS induction compared with that in the control. The phosphorylation level increased after VEGF treatment. In conclusion, VEGF is a critical mediator involved in functional recovery after spinal transection and can be considered a potential target for clinical therapy.

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Poster

051. Axon Injury and Recovery

Location: Hall A

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation
European Research Council (ERC) advanced grant (Nogorise)
Christopher and Dana Reeve Foundation
Swiss Continence Foundation
Santa Casa da Misericórdia de Lisboa (Prémio Mello e Castro- 2016)

Title: Spinal circuits involved in the lower urinary tract function of the rat

Authors: *A. M. SARTORI[1,2], A.-S. HOFER[1], C. D. CRUZ[3], T. M. KESSLER[2], M. E. SCHWAB[1];
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Abstract: Storage and voiding of the urine are achieved by complex interactions between the somatic and the autonomic nervous system. Although the storage reflex is mainly an intraspinal process, the initiation of voiding depends on supraspinal inputs from the pontine micturition center, which sends long-projecting axons to the spinal cord. After a suprasacral spinal cord injury (SCI), the voluntary control of micturition is disrupted, leading to detrusor overactivity and detrusor-sphincter-dyssynergia (DSD). Up to date, the connectivity changes responsible for the development lower urinary tract dysfunction after SCI are unknown. Thus, we performed anatomical analyses of the lumbosacral cord of rats that received a thoracic SCI at different time-points after injury. Rats were implanted with a tubing system for controlled bladder filling and monitoring of bladder pressure, and external urethral sphincter (EUS) electromyography electrodes, allowing for repetitive urodynamic measurement and recording of EUS activity in awake animals over time. A severe but incomplete spinal cord injury was induced in 16 animals at the thoracic level 8, while 5 rats received a sham surgery. Urodynamic investigations were continuously performed for 2h at 7, 16, and 28 days after SCI just prior to euthanasia. Thereafter, the anatomical changes in the spinal cord were analysed. Urodynamics confirmed an areflexic detrusor and EUS muscle during the first week after injury. Starting from week 2 after injury, bladder function reappeared but it was inefficient due to DSD, as confirmed by urodynamic parameters showing the simultaneous contraction of the EUS and the detrusor muscle. The 2h urodynamic investigations allowed the activity marker c-Fos to be sufficiently expressed in cells involved in lower urinary tract function, thus permitting the localization and characterization of these spinal neurons. Quantification of the different cell populations in the lumbosacral cord using immunohistochemistry and *in-situ* hybridization is currently ongoing.

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Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.18/H4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA Grant I01BX007080

Title: Spike timing-dependent plasticity in the adult rat with chronic cervical spinal cord contusion

Authors: *N. DE LA OLIVA[1], A. E. HAGGERTY[1], M. A. PEREZ[1,2], M. OUDEGA[1,2,3,4]; [1]Miami Project To Cure Paralysis, Univ. of Miami, Miami, FL; [2]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL; [3]Affiliated Cancer Hosp. & Inst. of Guangzhou Med.

Univ., Guangzhou, China; [4]Neurolog. Surgery, Miller Sch. of Medicine, Univ. of Miami, Miami, FL

Abstract: Spinal cord injury (SCI) damages descending and ascending axons resulting in motor and sensory function impairments. Histological and electrophysiological data revealed that in most SCI patients residual axonal connections between the brain and the spinal cord below the injury exist, which opens avenues for neuromodulatory therapies for recovering function. Electrical stimulation of residual axonal connections is a promising strategy to recover lost function. Repetitive electrical stimulation results in persistent increase or decrease of synaptic efficacy (i.e., long-term potentiation or depression, respectively). Previous studies demonstrated that the arrival of repeated pairs of precisely timed presynaptic and postsynaptic action potentials to a given synapse changes synaptic strength. This process is known as spike timing-dependent plasticity (STDP). The direction of the effects of STDP stimulation protocols depends on the spike order and time between the central and peripheral stimuli, as well as on the frequency and duration of the stimulation. Importantly, it was shown that STDP protocols can enhance motor function after paired corticospinal tract (CST) and peripheral nerve stimuli in people with and without SCI, although with transient effects. In this study, we aimed to elucidate the cellular and molecular mechanisms underlying STDP aftereffects in a cervical SCI rat model. First, we traced the CST and the reticulospinal tract (RST), which are both involved in forelimb reach and grasp behavior in rats, along with the motoneurons of targeted forelimb muscles, to evaluate the spinal connections before and after 12 weeks C5 chronic injury. Based on these results, an STDP stimulation protocol was applied to maximize the synaptic strength in those connections. Electrophysiological and histological techniques were used to evaluate changes after the stimulation. We hypothesize that higher frequency and longer stimulation will result in longer-lasting functional and cellular aftereffects. The ultimate goal is to use the data from our animal studies to improve the efficacy of STDP protocol on improving function in SCI patients.

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Poster

051. Axon Injury and Recovery

Location: Hall A

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Program #/Poster #: 051.19/H5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NINDS 5F32NS096883

Title: Spleen tyrosine kinase promotes neutrophil activation and exacerbates long-term neurologic deficits following spinal cord injury

Authors: *D. A. MCCREEDY, C. L. ABRAM, Y. HU, C. A. LOWELL;
Univ. of California, San Francisco, CA

Abstract: The acute phase of spinal cord injury (SCI) results in rapid and extensive accumulation of neutrophils, however, the direct impact of neutrophils on acute tissue damage and long-term recovery remains unclear. Spleen tyrosine kinase (Syk) is a prominent intracellular signaling mediator that has been shown to facilitate the development of neutrophil effector functions in response to inflammatory stimuli, but has not been studied in the context of SCI. To determine the role of Syk in neutrophils after SCI, we bred $Syk^{f/f}$ with MRP8-Cre mice to produce a neutrophil specific deletion of Syk. Contusive SCI was performed in adult male and female $Syk^{f/f}$ control and $Syk^{f/f}$MRP8-Cre littermates at thoracic vertebral level 9 (T9) using a previously validated weight drop method (2g dropped from 7.5cm). Researchers were blinded to genotype and Basso Mouse Scale (BMS) testing was performed at 1, 3, and 7 days post-SCI and weekly thereafter for 5 weeks. By 3 weeks post-SCI, we observed improved BMS scores in $Syk^{f/f}$MRP8-Cre mice relative to $Syk^{f/f}$ mice. Improved BMS scores were maintained through the end of the study at week 5. To determine the effect of $Syk^{f/f}$MRP8-Cre in acute inflammation, we performed flow cytometry at 24 hours post-SCI to identify myeloid cells, including neutrophils and monocytes. While no differences were observed in the accumulation of myeloid cells into the injured spinal cord, higher L-selectin levels were observed on infiltrated neutrophils in the spinal cords of $Syk^{f/f}$MRP8-Cre mice compared to $Syk^{f/f}$ control mice, indicative of reduced activation. No differences in L-selectin were observed on circulating myeloid cells or on infiltrated monocytes. These data suggest that Syk may contribute to hyper-activation of neutrophils in the injured spinal cord. Furthermore, we observed reduced expression of pro-inflammatory genes CSF1 and iNOS in infiltrated neutrophils isolated from the spinal cords of $Syk^{f/f}$MRP8-Cre mice. There was also a strong trend towards reduced $CitH3^{+}$ neutrophils in the spinal cord of $Syk^{f/f}$MRP8-Cre vs. $Syk^{f/f}$ mice, suggesting reduced neutrophil extracellular trap formation in the absence of Syk. Our findings implicate Syk expression in neutrophils as a novel mediator of acute inflammation and long-term neurologic deficits after SCI.

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Poster

051. Axon Injury and Recovery

Location: Hall A

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: R21NS106309
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R01NS083983

Craig Nielsen Post doc fellowship (Venkatesh)

Title: An integrated *in silico* pipeline identifies a novel TF combination that promotes enhanced CST growth following injury

Authors: *I. VENKATESH[1], Z. WANG[2], V. MEHRA[1], E. EASTWOOD[1], M. SIMPSON[1], A. CHAKRABORTY[1], D. GROSS[1], Z. BEINE[1], M. CABAHUG[1], G. OLSON[1], M. G. BLACKMORE[3];
[2]Dept. of Biomed. Sci., [3]Biomed. Sci., [1]Marquette Univ., Milwaukee, WI

Abstract: Embryonic and peripheral neurons respond to axonal injury with activation of transcriptional networks conducive to re-growth. In contrast, injured mature CNS neurons fail to re-induce appropriate transcriptional networks, resulting in failed regeneration and permanent damage. We have previously shown that forced re-expression of single transcription factors (TFs) such as KLF6 promotes axon outgrowth following injury and is a promising strategy for therapeutic neural repair. However, with single TF treatments the overall number and regenerative speed of axons remains sub-optimal, and likely insufficient for full functional recovery. Because TFs rarely function in isolation, we hypothesized that supplying combinations of TFs that synergize with KLF6 may boost growth phenotypes. To this end, we developed a bioinformatics pipeline to detect TFs that may synergize with KLF6 to drive the expression of pro-growth genes. To validate our bioinformatic predictions, we next systematically co-expressed candidate TFs in combination with KLF6 in assays of neurite outgrowth in post-natal CNS neurons. Remarkably, nearly 20% of the TF synergized with KLF6 to promote neurite outgrowth, validating the bioinformatics approach. To prioritize TFs for *in vivo* testing, we performed TF - target gene network analyses that identified 4 core TFs - EOMES, RARB, NKX32 and NR5A2 that are predicted to be critical to the regulation of pro-growth gene networks. Finally, we tested *in vivo* the ability of the 4 core TFs to promote CST axon growth individually or in combination with KLF6 following pyramidotomy injuries. Individually, RARB overexpression lead to increased CST sprouting following pyramidotomy. Importantly, we observed that although NR5A2 had no effect on growth by itself, forced co-expression with KLF6 promoted a robust increase in midline crossing by transduced CST axons, significantly above the level of KLF6 alone. Ongoing experiments are aimed at clarifying the molecular mechanisms underlying KLF6/NR5A2 synergy in driving axon growth. Overall, we describe a novel bioinformatics-based approach that has identified a completely novel TF combination that drives enhanced sprouting in the injured CST.

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Poster

051. Axon Injury and Recovery

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R21AG057974
NIH-ENDURE Program (R25 NS080268706)
NIH-MBRS RISE Program (5R25GM061151-17)

Title: Characterization of radial nerve cord *in vitro* explants of sea cucumber Holothuria glaberrima

Authors: *R. A. GARCIA ROSARIO[1], A. J. SIRFA LOPEZ[2], E. A. QUESADA DIAZ[1], P. V. FIGUEROA DELGADO[1], J. E. GARCIA ARRARAS[1];
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Abstract: The sea cucumber *Holothuria glaberrima* is a marine invertebrate with the capacity to regenerate its central nervous system (CNS). As an echinoderm, this organism is closely related to vertebrates. Its CNS is composed of an anterior circumoral nerve ring from which five radial nerve cords (RNCs) extend posteriorly. The RNCs are ganglionated nerves with neuronal somas in the periphery whose fibers form a neuropile in the center of the structure. Radial glia are interspersed among the neurons with elongations from the basal to apical ends. Unlike the mammalian CNS, this animal model is capable of undergoing a fast, efficient and scar-less CNS regeneration following injury. We have now developed an *invitro* system where to determine the cellular and molecular mechanisms of RNC regeneration. This system provides a better controlled physical-chemical environment for pharmacological and molecular studies. The RNCs together with the surrounding tissues were dissected and treated with collagenase to separate them from the longitudinal muscles and body wall. They were placed in culture with high aseptic techniques and a supplemented medium and left for up to 11 days in culture (dc). Explants maintained their structure in culture although they tended to curve and join their stumps after 3 dc. We manually counted and developed RNC surface plots to determine cell nuclei presence by regions. The number of cells within the RNC did not change significantly, although their distribution was altered by cell movement from the periphery into the neuropile region. Radial glial cells seemed to undergo a dedifferentiation process, losing most of their radial projections. Little or no proliferation was detected using BrdU assay. Similarly, little apoptosis was detected during the first week in culture but increased lightly at 11 dc. Our results serve to establish the basis for the cellular mechanisms that RNCs undergo under culture conditions. Thus, this study

provides a unique tool for further understanding of how the regenerative process occurs and why it is limited in vertebrates.

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Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.22/H8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation Grant 457328
WIngs for Life grant WfL-DE-006/12
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NIDILRR Grant 90SI50200100

Title: Acquired infections impair recovery after spinal cord injury

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Abstract: Patients become severely immunocompromised after spinal cord injury (SCI), making them vulnerable to infections. One of the most severe of these infections is pneumonia. These infections are the leading cause of death after SCI. Additionally, patients that acquire infections have reduced recovery potential compared to SCI patients without infections. We have developed a translationally relevant model to study the effects of acquired infections after SCI. Mice with acquired infections have impaired functional recovery after SCI compared to SCI mice without infection. Using immunohistochemistry we have identified some of the underlying effects leading to functional impairment after infection. Together this model can be used to develop future therapies for SCI patients in order to preserve rehabilitation potential.

Disclosures: A.R. Filous: None. **J.M. Schwab:** None.

Poster

051. Axon Injury and Recovery

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Program #/Poster #: 051.23/H9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: MCFP
Bluepharma
RESOLVE

Title: Profilin1 delivery tunes cytoskeleton dynamics towards CNS axon regeneration

Authors: *R. PINTO-COSTA[1], S. C. LEITE[2], M. M. SOUSA[1];
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Abstract: After trauma, regeneration of adult CNS axons is abortive causing devastating neurologic deficits. Despite progress in rehabilitative care, there is no effective treatment stimulating axon growth following injury. Using models with different regenerative capacities, followed by gain- and loss-of-function analysis, we identified profilin1 (Pfn1) as a coordinator of actin and microtubule (MT) dynamics in growth cones, powering axon growth and regeneration. Molecularly, we further show that in neurons, Pfn1 enhances MT dynamics through a formin-mediated mechanism rather than by directly interacting with MTs. *In vitro*, increased Pfn1 activity was instrumental for orchestrating cytoskeleton dynamics towards axon growth. *In vivo*, Pfn1 ablation limited regeneration of growth-competent axons after spinal cord injury. Conversely, adeno-associated viral (AAV) delivery of constitutively active Pfn1 in adult rats promoted axon regeneration of injured sciatic nerves and of transected ascending sensory spinal cord tracts. This indicates that modulation of Pfn1 levels and activity is instrumental to successfully achieve a regenerative phenotype. Our findings suggest new therapeutic strategies to promote axon regeneration by targeting the activity of regulators of actin dynamics. Specifically, AAV-mediated delivery of constitutively active Pfn1, together with the identification of modulators of Pfn1 activity, should be considered for the treatment of the injured nervous system.

Disclosures: R. Pinto-Costa: None. **S.C. Leite:** None. **M.M. Sousa:** None.

Poster

051. Axon Injury and Recovery

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Fondo de Investigacion Anahuac Mexico

Title: Analysis of metabolic, inflammatory, motor and neurological changes of obesity in an *in vivo* spinal cord injury model

Authors: O. OJEDA- GONZALEZ, ***R. H. RODRIGUEZ BARRERA**, A. FLORES-ROMERO, E. GARCIA- VENCES;
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Abstract: Nowadays, obesity (OB) is a very important condition due to its rapid rise in prevalence and incidence; in accordance with ENSANUT 2016, the prevalence of overweight in Mexico is about 70%, becoming the main cause of several diseases due to metabolic and inflammatory complications. Spinal cord injury (SCI) produced metabolic and inflammatory changes in both systemic and at the lesion site. The goal was to evaluate metabolic, inflammatory, motor and neurologic changes in obese rats after SCI. Forty rats randomly were allocated into 4 groups (Groups: 1. Control; 2. OB; 3. OB+SCI and 4. SCI ; n=10 each group). The experimental design was carried out in two phases: First, rats were exposed to high-fat diet to get diet-induced obesity and weight and body mass index (BMI) was evaluated in comparison with the standard-diet control group. In second phase, rats were subjected to a moderate SCI and the blood samples were collected (before injury and thirty and sixty days after injury) to evaluate glucose, serum total protein, triglycerides, total cholesterol, insulin and proinflammatory cytokines (IL-6, IL-1β and TNF-α) levels. Also, locomotor recovery was assessed from SCI at sixty days after it. Our results showed a significant difference in the weight and BMI between high-fat diet and control group (p=0.0001). Insulin, triglycerides, serum total protein and IL-6 levels were significant different sixty days after injury in the OB+SCI vs SCI group. Meanwhile there were no differences in glucose, total cholesterol, motor recovery, TNF-α and IL-1β. In conclusion, the type of high-fat diet is essential to establish a successful diet-induced obesity model. We suggest that locomotor recovery does not depend on previous body condition. The serum total protein decrease suggests a protein catabolism in OB+SCI group. Hypertriglyceridemia in OB+SCI could be explaining by the type of diet administered and the hypertrophy and hyperplasia of adipocytes. High levels of IL-6 play an important role in proinflammatory state; however, other inflammation indicators must be studied.

Disclosures: O. Ojeda- Gonzalez: None. **R.H. Rodriguez Barrera:** None. **A. Flores-Romero:** None. **E. Garcia- Vences:** None.

Poster

051. Axon Injury and Recovery

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01NS110385
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5 R01 MH100093-05

5R01DA022727-11
1R01NS106906-01

Title: Modulating the EphB2-NMDA receptor interaction in the superficial dorsal horn attenuates neuropathic pain following cervical spinal cord injury

Authors: *N. M. HEINSINGER, W. ZHOU, J. L. WATSON, A. FALNIKAR, T. FOX, E. V. BROWN, B. A. CHARSAR, M. B. DALVA, A. C. LEPORE;
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Abstract: In a rodent model of cervical spinal cord injury (SCI), we examined the contribution of altered EphB2 receptor-NMDA receptor (NMDAR) interaction to both excitatory synaptic neurotransmission in the superficial dorsal horn (DH) and persistent neuropathic pain (NP). The development of NP occurs in a significant portion of individuals affected by SCI, resulting in debilitating and often chronic physical and psychological burdens. Importantly, this pathological pain is particularly refractory to treatment, urgently calling for the identification of mechanistic targets that both robustly regulate pathological pain and avoid the devastating effects of opioid based interventions. Hyperexcitability of DH circuitry ("central sensitization") is a major substrate for NP after SCI. Studies have shown that NP is linked to EphB/ephrinB signaling through potentiation of NMDAR function, suggesting that the EphB-NMDAR interaction may be an important target for control of SCI-induced NP. In particular, we previously reported that EphB2 activation stimulates a direct interaction between EphB2 and the NMDAR via a single extracellular amino acid of EphB2 (Y504), thus promoting NMDAR synaptic localization and excitatory synapse function. Using a rodent model of unilateral cervical contusion SCI, we observe a persistent at-level NP phenotype in the form of forepaw thermal hyperalgesia and mechanical allodynia. We find that EphB2 expression is upregulated in superficial lamina of the ipsilateral DH after cervical contusion in intact cervical regions caudal to the lesion (i.e. at the location of primary afferent input from the plantar surface of the forepaw). Confocal imaging reveals significantly increased co-localization of EphB2 and the GluN1 NMDAR subunit at vGlut1-positive synaptic sites in superficial DH neurons after cervical contusion, indicating an enhanced interaction between EphB2 and the NMDAR at putative excitatory glutamatergic synapses. Lastly, lentiviral shRNA knockdown of EphB2 via anatomically-targeted intraspinal DH injections performed at 7 days post-cervical contusion reversed already-established thermal hyperalgesia. Collectively, these findings suggest that enhanced EphB2 expression and EphB2-NMDAR interaction underlies alterations in excitatory synaptic transmission in the DH and consequently persistent NP following SCI.

Disclosures: **N.M. Heinsinger:** None. **W. Zhou:** None. **J.L. Watson:** None. **A. Falnikar:** None. **T. Fox:** None. **E.V. Brown:** None. **B.A. Charsar:** None. **M.B. Dalva:** None. **A.C. Lepore:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.26/H12

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Systemic protein kinase inhibition reduces local inflammation after cervical spinal cord injury

Authors: *M. ZAVVARIAN[1], J. HONG[1], M. KHAZAEI[2], J. WANG[3], M. G. FEHLINGS[4]; [1]Univ. of Toronto, Toronto, ON, Canada; [2]Dept. of Genet. and Develop., Krembil Res. Institute, Univ. Hlth. Netw, Toronto, ON, Canada; [3]Univ. Hlth. Network, Toronto, ON, Canada; [4]Div. Neurosurg., Toronto Western Hosp., Toronto, ON, Canada

Abstract: Traumatic spinal cord injury (SCI) is a life-threatening and multifaceted condition that prompts permanent disability for millions of patients worldwide. The disruption of blood-spinal cord barrier (BSCB) by physical trauma is a major challenge in SCI treatment, as it results in the infiltration of reactive immune cells that cause further secondary damage to the spinal cord. Therapeutic stabilization of BSCB can potentially attenuate the immune cells migration and improve SCI recovery. The purpose of this study is to examine the effects of systemic protein kinase inhibition on BSCB integrity, and to determine its efficacy as a treatment for SCI. It is hypothesized that midostaurin—a clinically approved protein kinase inhibitor—mitigates the secondary SCI pathogenesis by reducing immune cells migration. To this aim, clip-compression SCI model at C6-7 was used to assess the effects of intraperitoneal midostaurin injection (25 mg/kg) in Wistar rats. The results suggest that midostaurin inhibits GSK3β phosphorylation in the injury epicenter. This leads to downregulation of adhesive and migratory genes including JAM2, THY1 and ITGB1, and ultimately reducing proinflammatory markers, such as fractalkine, IL-1a, and IL-5 at 1-day post-injury. This study demonstrates that systemic protein kinase inhibition is an effective strategy for preventing secondary SCI damage, which can have a significant impact on the enhancement of neuroprotective regime applied upon traumatic SCI.

Disclosures: M. Zavvarian: None. **J. Hong:** None. **M. Khazaei:** None. **J. Wang:** None. **M.G. Fehlings:** None.

Poster

051. Axon Injury and Recovery

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Program #/Poster #: 051.27/H13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS R01NS091582
NINDS T32 NS077889
NINDS F31 NS105443

Title: Myelin modulates macrophage inflammatory responses after spinal cord injury

Authors: *T. J. KOPPER[1], K. B. BETHEL[2], B. ZHANG[1], W. M. BAILEY[1], J. C. GENSEL[1]; [1]Physiology, Spinal Cord and Brain Injury Res. Ctr., [2]Neurosci., Univ. of Kentucky, Lexington, KY

Abstract: Spinal cord injury (SCI) produces chronic inflammation largely mediated by resident microglia and infiltrating monocytes (here, collectively referred to as macrophages). These activated SCI macrophages eventually adopt a pro- inflammatory, pathological state that continues long after the initial injury. Pro-inflammatory macrophages potentiate secondary damage and impair SCI recovery, yet the mechanisms driving chronic pathological SCI macrophage activation are poorly understood. After SCI, macrophages clear and accumulate extensive myelin debris. Published data demonstrates that myelin debris can directly stimulate macrophages to adopt different activation states. We hypothesize that myelin, in combination with inflammatory stimuli within the SCI lesion environment, increases pro-inflammatory macrophage activation.

To test this hypothesis we stimulated bone marrow derived macrophage with pro-inflammatory stimuli (LPS+INF-γ) *in-vitro* in the presence or absence of myelin. Myelin co-stimulation significantly increased pro-inflammatory IL-12 cytokine production, decreased anti-inflammatory IL-10 production, and increased reactive oxygen species and nitric oxide production relative to unstimulated or LPS+INF-gamma treated controls. We hypothesize that this myelin-mediated potentiation of pro-inflammatory macrophage activation is dependent on the enzyme cytosolic phospholipase A2 ($cPLA_2$). This enzyme has the potential to modify membrane lipids into direct and indirect pro-inflammatory stimuli. Indeed, inhibition of $cPLA_2$ *in-vitro* was seen to block the detrimental effects of myelin on pro-inflammatory macrophages. Further, immunohistochemical analyses of spinal cord tissue sections after T9 contusion SCI in female C57BL/6 mice indicate $cPLA_2$ activation in myelin-laden macrophages at both 7 and 28 days post injury. Ongoing studies aim to specifically target this macrophage $cPLA_2$ activity after SCI.

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Poster

051. Axon Injury and Recovery

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Topic: C.11. Spinal Cord Injury and Plasticity

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1 F31 NS106742-01

Title: DLK and LZK direct diverse responses to axon damage in larval zebrafish

Authors: *K. P. ADULA[1], H. MARKOVIC[1], A. SAGASTI[2];
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Abstract: Dual leucine kinase (DLK), a Map kinase kinase kinase, is a conserved axon damage sensor in worms, flies and mice. Vertebrates have two DLK-related genes, DLK and leucine zipper kinase (LZK). To study the individual contributions of DLK and LZK in a tractable vertebrate model, I made null mutants of both genes in zebrafish, using the CRISPR/Cas9 system. Larval zebrafish are an excellent system in which to study axon regeneration, since both the central and peripheral environments are permissive and the dynamics of axon regrowth can be tracked in live animals. Single and double DLK/LZK mutants reached adulthood, and their larval neurons appeared to develop normally. To assess regeneration in these mutants, I mosaically expressed neuronal reporters to label single cells for 2-photon laser axotomy, and tracked each individual cell before, during, and after axotomy. In DLK and LZK single mutants, motor neurons regenerated normally, but regeneration in double mutants was significantly impaired, indicating that DLK and LZK are redundantly required for motor axon regeneration. To determine if these proteins may have compartment-specific roles in axon regeneration, I separately assessed regeneration of the central and peripheral axons of Rohon-Beard (RB) somatosensory neurons. I performed RB axotomies either at the primary branch of RB neurons (leaving no spared branches), or at a secondary branch, which allowed me to distinguish regeneration at the cut axon stump from collateral sprouting at the spared branch. Central axons of DLK and LZK mutants regenerated similar to, or slightly better, than wildtype RB axons. Surprisingly, peripheral axons of RB neurons regenerated more robustly in DLK, LZK and DLK/LZK double mutants. Excessive growth following axotomy was more pronounced from spared branches than from the axon stump. These observations indicate that, contrary to their function in motor neurons, DLK and LZK limit axon growth after RB neuron injury. Rescue experiments are underway to determine whether these divergent outcomes are due to cell autonomous or non-cell autonomous DLK or LZK functions. Together, these results suggest that,

like in other systems, DLK and LZK regulate axon regeneration in zebrafish, but have evolved different roles in different cell types: redundantly promoting axon regeneration in motor neurons, and restricting growth in somatosensory neurons.

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Poster

051. Axon Injury and Recovery

Location: Hall A

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Topic: C.11. Spinal Cord Injury and Plasticity

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Title: Molecular and histologic outcomes indicate reduced wounding following spinal cord injury in spiny mice, Acomys cahirinus

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Abstract: Injury to the mammalian spinal cord results in neurological impairments due to activation of immune cells (i.e., astrocytes, macrophages, microglia) and subsequent fibrosis and scar formation. The adult African spiny mouse (*Acomys cahirinus)* shows little to no local scarring or fibrosis after skin or muscle injury, a response unique among mammals. Here we explored the *Acomys* molecular and histologic response to cervical spinal cord injury (SCI). We hypothesized that *Acomys (n=15)* would have reduced spinal fibrosis and immune response to C3/C4 hemi-crush injury (C3Hc) as compared to C57BL/6 mice (*Mus; n=16*). Evaluation of the cervical spinal cord at 3-days post-injury using pathway-focused mRNA gene arrays revealed striking differences between the two groups. A “wound healing” array indicated a stronger response in *Mus* (16 upregulated genes) *vs*. *Acomys* (3 upregulated genes). Conversely, a “neurogenesis” array showed a stronger response in *Acomys* (22 upregulated genes) as compared to *Mus* (7 upregulated genes). Histological evaluation of the spinal lesion also indicated a differential response in *Acomys* vs. *Mus.* At 4-wks post-injury, *Acomys* had reduced immunostaining for a marker associated with spinal scarring (collagen IV; $p<0.0001$ vs. *Mus*). Additionally, there was a trend towards reduced astrocyte (glial fibrillary acidic protein, GFAP)

and macrophages/microglia, (ionized calcium binding adapter molecule 1, Iba1) immunostaining, but did not reach statistical significance (GFAP: p=0.147; Iba1: p=0.085). This molecular and histologic evaluation indicates that *Acomys* has a different local tissue response to SCI as compared to *Mus*. The reduced inflammation and fibrosis coupled with increased activation of regenerative pathways indicates that *Acomys* may be a useful comparative model to study adaptive responses to SCI.

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Poster

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Shriners Hospital for Pediatric Research SHC 84051
Shriners Hospital for Pediatric Research SHC 86000
Department of Defense SC140089

Title: Viral expression of constitutively active AKT3 induces CST axonal sprouting and regeneration, but also promoted seizure activity

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Abstract: The regeneration of severed axons, particularly descending motor tracts, in the central nervous system (CNS) has long been the goal of those studying spinal cord injuries (SCI). To date, a number treatments have shown some regeneration and/or sprouting within the spinal cord. One of the most studied pathways involved in the potentiation of axonal regeneration is the PI3K/AKT/mTOR pathway. It is known that intervention within this pathway can have profound effects on the growth and injured axons. Here we examined if AKT3, a serine/threonine protein kinase, can induce regeneration of injured axons. It has been previously shown that the overexpression of signal transducer and activator of transcription 3 (STAT3) was able to boost axonal regeneration and sprouting in the injured CNS. While both AKT3 and STAT3 are able to contribute to the regeneration of axons independently, the aim of this study was to observe their effects on the corticospinal tract (CST) in conjunction with one another. To determine CST regeneration and sprouting, we induced expression of constitutively active (ca) AKT3, caSTAT3,

or both by injecting Adeno-Associated Viral (AAV) vectors into the somatosensory forelimb cortex of the brain of adult rats. Two weeks later, the CST was severed via a dorsal column lesion (DCL) at the C5 level of the spinal cord. All animals were examined for functional recovery using single pellet reaching and IBB forelimb assessment. Two weeks prior to the end of the study, rats were injected with an anterograde tracer, biotinylated-dextran amine (BDA) in the same somatosensory coordinates as the initial AKT3/STAT3 vector induction to label axons of the CST. Our results show that the expression of caAKT3, caSTAT3, or both in the injured CST work synergistically to promote an increase of axonal regeneration when compared to controls, with the combination of caAKT3 and caSTAT3 showing the best sprouting and regeneration of the CST. Unfortunately, none of these groups showed a statistically significant increase in behavioral recovery, with some showing decreased function. Interestingly, the majority of animals with injections of caAKT3 displayed various levels of seizure activity, ranging from mild to very severe. These rats also showed an enlarged cortical hemisphere with very large neurons reminiscent of hemimegaloencephaly.

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Poster

051. Axon Injury and Recovery

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD Award: W81XWH-17-1-0629

Title: Effects of morphine on reactive microglia and macrophages in an SCI rodent model

Authors: *M. N. TERMINEL[1,2], J. A. RAU[1,2], K. BRAKEL[1,2], R. DUNDUMULLA[1], M. A. HOOK[1,2], C. RUIZ[1];
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Abstract: Opioids are among the most effective and commonly prescribed analgesics for the treatment of acute pain after spinal cord injury (SCI). We have shown, however, that morphine administration in the early phase of SCI undermines locomotor recovery in a rodent contusion model. Based on our previous studies we hypothesize that morphine acts on classic opioid receptors to alter the immune response after SCI. Our data indicate that morphine increases the expression of immune cells after SCI. However, in-vitro studies also suggest that morphine can reduce the phagocytic activity of these cells, potentially contributing to the increase in cell death we have observed. Whether morphine increases or decreases the immune response after SCI

remains unclear. To test this, we assessed whether morphine affects the immune response by altering the temporal expression of microglia and macrophages (Exp. 1) or by changing the innate function of these cells (Exp. 2). Methods: Subjects were given a moderate spinal contusion injury. On the day following surgery, half of the subjects were treated with morphine (i.v.) for 1, 3, or 7 days. The remaining subjects served as controls, receiving an equivalent volume of 0.9% saline. Subjects were euthanized on days 2, 4 or 8 (24 hrs after the final dose of morphine). A 1.5 cm section of the injured spinal cord was collected and the tissue was enzymatically and mechanically dissociated. Cells were incubated with antibodies to identify macrophage and microglia populations. Additional antibodies were included to differentiate between the M1 (pro-inflammatory) and M2 (anti-inflammatory) cells. For exp. 2, we performed an ex-vivo phagocytic assay on SCI microglia and macrophages. CD11b+ cells were extracted from primary dissociated cells using magnetic cell sorting, then incubated for 2 hours with FITC-beads, and subsequently analyzed with flow cytometry. Results: Higher numbers of macrophages and microglia can be detected as early as 3 days after morphine administration. Our results also suggest that morphine increases both M1 and M2 immune cells. However, despite this increase, we found that phagocytic activity of M1 macrophages is decreased after 3 days of morphine administration. Microglia phagocytic activity remained unaffected.
Conclusion: Further studies are needed to explore whether reduced macrophage phagocytic activity affects microglia function and increases cell death. Given the clinical utility of opioid analgesics, it is imperative that we fully understand the effects of morphine in mediating the immune response after SCI. We must develop safe and effective therapeutic strategies for the use of opioids in pain management after SCI.

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Poster

051. Axon Injury and Recovery

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig Neilsen Postdoctoral Fellowship Award #337416
NIH Grant 1NS082446
U of Missouri SCIDRP

Title: Assessment of the growth competence of ascending sensory neurons after spinal cord injury

Authors: *E. EWAN, D. CARLIN, G. ZHAO, V. CAVALLI;
Dept. of Neurosci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Spinal cord injury (SCI) damages long projecting axons leading to loss of sensory and motor function. Many studies indicate that unlike peripheral nerve injury, SCI fails to activate a pro-regenerative program in dorsal root ganglion (DRG) neurons, which fail to regenerate their axons after SCI. Previous studies analyzed whole lumbar DRG after SCI, but only a small portion of DRG neurons ascend the spinal cord and are injured after SCI. In addition, neurons account for only ~10% of all cells within the DRG. We thus sought to assess the effects of SCI specifically in DRG neurons whose axons ascend the spinal cord dorsal column. We first confirmed that the YFP16 transgenic mouse line expresses YFP specifically in dorsal column neurons (proprioceptors and low threshold mechanoreceptors (LTMRs). Next, we found that the regeneration-associated genes ATF3 and Jun are upregulated specifically in a subset of YFP positive neurons after thoracic SCI, suggesting that some DRG neurons may be primed into a pro-growth state after SCI. To evaluate this possibility, we performed an *in vitro* conditioning experiment and found that SCI did not promote *in vitro* growth of YFP positive neurons. While this result suggests that ATF3 and Jun alone are not sufficient to activate a growth program in DRG neurons after SCI, it reveals that DRG neurons do respond to SCI by changing their gene expression. Thus, we next analyzed gene expression of L4 YFP positive neurons after thoracic SCI or sciatic nerve crush injury (SNC) by FACS-RNAseq of YFP positive L4 DRG neurons. As expected, we found that more genes were differentially expressed after SNC than SCI, including many ion channels. Furthermore, upregulation of many known regeneration-associated genes was less after SCI compared to SNC. Specifically, hypoxia-inducible factor 1α (HIF-1α), which we previously shown is both necessary and sufficient to promote peripheral nerve regeneration, is upregulated after SNC but not SCI in YFP positive neurons. We next tested the efficacy of pharmacological HIF-1α stabilization in promoting axon regeneration in a CNS culture model and identified two compounds that increase regeneration in cortical neurons. We are currently investigating whether pharmacological or environmental (acute intermittent hypoxia) stimulation of neuronal HIF-1α promotes axon regeneration after SCI, and whether manipulating any of the ion channels shown to be differentially regulated between SNC and SCI contribute to axon regenerative capabilities of injured sensory neurons.

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Poster

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH 5T32 NS077889

Title: A novel mammalian model of spinal cord injury responses and repair: The African spiny mouse (Acomys cahirinus)

Authors: *M. ORR[1], W. BAILEY[1], C. CALULOT[1], K. MCFARLANE[1], S. KAPP[2], K. RICHARDS[2], A. SEIFERT[3], J. GENSEL[1];
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Abstract: Current mammalian models of spinal cord injury (SCI) recapitulate human injury responses and result in poor regeneration of spinal tissues. Neonatal and non-mammalian models of spinal regeneration have been used to identify pathways toward inducing regeneration, but the translation of these targets to adult mammals, especially humans, is extremely difficult. Therefore, an adult mammalian model with unique injury responses and regenerative potential would serve as a powerful tool in identifying translational regenerative therapeutic targets. African spiny mice (genus *Acomys*) are rodents with extensive regeneration of full-thickness skin and associated glands, adipose tissue, cartilage, muscle, kidneys, and nerves after injury. *Acomys* multi-organ regeneration is coincident with inflammatory and fibrotic responses distinct from the non-regenerating laboratory mouse (genus *Mus*). While the inflammatory and fibrotic responses to injury are known to influence the progression of SCI damage or repair, there are no published studies of *Acomys* regenerative capacity in the spinal cord. The objective of this study is to evaluate the *Acomys* injury responses and recovery from SCI, thereby identifying its efficacy as an adult mammalian model for translational SCI research. Through histological analysis of uninjured tissues, we found that *Acomys* and *Mus* have similar gross neuroanatomical spinal structures. However, histological analysis after SCI shows that *Acomys* deposit less extracellular matrix proteins and have a more specifically-localized inflammatory response than *Mus*. Furthermore, primary cell cultures show that these unique SCI response elements affect neuron growth and viability. Ongoing studies are evaluating if *Acomys* exhibit enhanced axon regeneration and functional recovery after SCI compared to poorly-regenerating *Mus*. Together, these results indicate that while *Acomys* have similar baseline spinal anatomy to *Mus*, their physiological response to SCI is unique and will likely affect the progression of SCI recovery. Moving forward, we will continue to assess the utility of the African Spiny Mouse (genus *Acomys*) as a novel mammalian model of SCI responses and repair.

Disclosures: M. Orr: None. **W. Bailey:** None. **C. Calulot:** None. **K. McFarlane:** None. **S. Kapp:** None. **K. Richards:** None. **A. Seifert:** None. **J. Gensel:** None.

Poster

051. Axon Injury and Recovery

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 051.34/H20

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Immunization with neural derived peptides induces neurogenesis in rats with chronic spinal cord injury

Authors: R. R. BARRERA, ***A. F. ROMERO**, E. G. VENCES, K. S. ZAVALA, L. N. TORRES, D. A. INCONTRI, J. W.-W. JUÁREZ, J. I. ARIAS;
Univ. Anáhuac, Estado de México, Mexico

Abstract: Background: Immunization with neural derived peptides (INDP) has demonstrated to be a promising therapy to reach a regenerative effect in the chronic phase of the spinal cord injury (SCI). In the present study, we analyzed the effect of the INDP in neurogenesis and function recovery, by BBB score, von Frey hair, double stain with 5-bromo-2'-deoxyuridine and doublecortin. Finally, examining the expression of inflammation-related and regeneration-associated proteins.
Results: The evaluation of motor recovery before therapeutic intervention showed that the BBB score was similar in both groups before treatments, although the rats in the INDP group had a significant increase in motor recovery. The mechanical hypersensitivity of the hind paw was measured by the withdrawal threshold mechanical stimulation with von Frey hair filaments. Similarly, INDP induced neurogenesis in rats with SCI. An ELISA assay determined that A91 immunization produced significant anti-inflammatory and regeneration-associated molecules in the chronic stages of the SCI. The rats also presented a significant increase of IL-4 and IL-10 and neurotrophic factors such as BNDF and GAP-43, in comparison with the PBS-immunized ones. The immunization with A91 induced a significant decrease in TNF-α, as expected.
Conclusions: These findings suggest that INDP could improve motor and sensitive recovery considerably in the chronic stage of SCI.
Key words: spinal cord injury, neurogenesis, protective autoimmunity, neural derived peptides.

Disclosures: R.R. Barrera: None. **A.F. Romero:** None. **E.G. Vences:** None. **K.S. Zavala:** None. **L.N. Torres:** None. **D.A. Incontri:** None. **J.W. Juárez:** None. **J.I. Arias:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.01/H21

Topic: D.03. Somatosensation – Pain

Support: WVSOM Intramural Grant

Title: Alterations in cerebellar BDNF and $MeCP_2$ expression in a repetitive acidic saline exposure persistent jaw muscle pain model

Authors: *J. MORRIS-WIMAN[1], C. G. WIDMER[2];
[1]Biomed. Sci., West Virginia Sch. of Osteo. Med., Lewisburg, WV; [2]Orthodontics, Univ. of Florida, Gainesville, FL

Abstract: Several imaging studies provide evidence that the cerebellum is activated with pain, but it remains unclear what role the cerebellum plays in nociceptive processing. Previously we assessed cerebellar expression of the pain-related factors, SP, CGRP and BDNF in a model for orofacial pain in which repetitive unilateral injections of acidic saline into masseter muscle elicits persistent bilateral pain. No differences were detected in either SP or CGRP immunolabeling within the cerebellum between pain and non-pain groups. However, significant differences were observed in immunolabeling for BDNF, with an increased expression in the pain group. This increase was prevented by APETx2, a ASIC3 blocker. BDNF expression is regulated by several epigenetic factors; the best characterized being the transcription modulator $MeCP_2$. However, it is not clear how precisely $MeCP_2$ regulates BDNF expression and there is conflicting data from different pain models as to whether BDNF expression is upregulated or downregulated by $MeCP_2$. The objective of this study was to determine if the observed increase in BDNF within the cerebellum in our persistent pain model was correlated with an increase or a decrease in $MeCP_2$ expression. **Methods**: Female CD-1 mice were repetitively injected with either neutral saline (pH 7, n=5), or acidic saline (pH 4, n=5) into the right masseter separated by five days. Five mice were injected with 10µl of APETx2 (3µM), into the right masseter just prior to the second acidic saline injection, 5 mice were used as unmanipulated controls. Seven days after the second injection, the mice were sacrificed and the cerebellum harvested. 12µm cryosections were immunostained for BDNF and $MeCP_2$ and images acquired using a Zeiss MRm digital camera and Axiovision software. Images were histogram-matched and thresholded to produce binary images to eliminate bias. Immunostaining for BDNF and $MeCP_2$ was assessed using an AOI outlining the granule and Purkinje cell layers and expressed as a percentage of the total area examined. **Results**: Significant differences were detected among groups in the relative immunostaining for both BDNF and $MeCP_2$ in both the Purkinje cell and granule cell layers. A significant increase for both BDNF and $MeCP_2$ was observed between the acidic saline-injected group and control, neutral and APETx2 groups (ANOVA, post-hoc LSD test, $p<0.05$). **Conclusions**: The results of this study indicate that the observed increased immunolocalization of BDNF in our persistent pain model is correlated with an increase in $MeCP_2$, suggesting that, in this model, $MeCP_2$ may have a role in activating BDNF expression and that this activation is prevented by blocking ASIC3.

Disclosures: J. Morris-Wiman: None. **C.G. Widmer:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.02/H22

Topic: D.03. Somatosensation – Pain

Support: JSPS KAKENHI 18K06884

Title: Involvement of melanopsin on the photic excitation of neurons in caudal trigeminal brainstem

Authors: *A. TASHIRO, Y. MORIMOTO;
Natl. Def. Med. Col., Tokorozawa, Japan

Abstract: Bright light can cause ocular discomfortand evokes protective reflexes such as blinking. Previously we reported that bright light activates trigeminal nerve activity through an intraocular mechanism driven by a luminance-responsive circuit (non-image visual functions) and increased parasympathetic outflow to the eye. Melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) are photoreceptors that mediate non-image visual functions.To determine if melanopsin activation was necessary for light-evoked Vc/C1 excitation, melanopsin antagonist (Opsinamides) was injected systemically while recording light-evoked Vc/C1 unit activity. Under isoflurane anesthesia, units were recorded in laminae I-II at Vc/C1 under low ambient light. All units received convergent mechanical input from the ocular surface and facial skin. Units with a cutaneous receptive field (RF) were tested for responses to pinch stimuli. Light stimulation (300W/m^2, 30s) was delivered from a thermal-neutral fiber optic source positioned 5 cm from the ocular surface at 20 min intervals. Light and mechanical evoked unit activity was recorded before and after 10 min after Opsinamides (1mg/kg, iv). Systemic administration of Opsinamides inhibited the light evoked unit activity. While, mechanical input from the convergent receptive field was not inhibited. In barbiturate anesthetized rats, Fos-like immunoreactive (Fos-LI) neurons at the Vc/C1 region were quantified after photic stimuli (300W/m^2, 30 s On/30 s Off, 30 min). Opsinamides or vehicle applied intravenously 10min prior to photic stimuli.Systemic administration of Opsinamides reduced light-evoked Fos-LI at the Vc/C1**.**These findings confirm that melanopsin plays a pivotal role in mediating light-evoked neural excitation at the Vc/C1 region. Activation of melanopsin may cause discomfort called dazzling sensation after intense light.

Disclosures: A. Tashiro: None. **Y. Morimoto:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.03/H23

Topic: D.03. Somatosensation – Pain

Support: NRF-2017R1A5A2015391

NRF-2017R1A2B2003561

Title: Synaptic connectivity of substance P-, CGRP-, isolectin B4-immunopositive axon terminals in the medullary dorsal horn in the normal rat and following inflammation

Authors: Y. CHO[1], J. LEE[1], H. HAN[1], D. AHN[2], H.-G. KO[1], *Y. BAE[1];
[1]Anat. and Neurobio., [2]Oral Physiol., Sch. of Dentistry, Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: Information on the synaptic connectivity of the peptidergic and nonpeptidergic C afferents in the medullary dorsal horn (MDH) in the normal condition and following inflammation may help understand how orofacial nociception conveyed via these C afferents is transmitted in the brain stem in normal and pathologic conditions. In this study, we investigated synaptic connectivity of the subsance P (SP)-, CGRP-, isolectin B4 (IB4)-immunopositive (+) axon terminals (boutons) in the superficial lamina of the MDH in naïve rat and following CFA application into the rat vibrissa pad by behavioral assay, serial section electron microscopy, immunocytochemistry and quantitative analysis. Most of the SP+ bouotns (~95%) and CGRP+ boutons (~80%) showed simple synaptic connectivity with one or two dendrites whereas large majority (~60%) of the IB4+ boutons showed complex synaptic connectivity with 3 or more upto 6 dendrites. IB4+ boutons frequently, but SP+ and CGRP+ bouotns never, received axoaxonic synapse implying presynaptic inhibition from glutamic acid decarboxylase+ axon terminals. Fraction of boutons forming synapse with dendritic spine of all SP+ or IB4+ boutons was significantly higher in the CFA-group showing mechanical allodynia than control. Number of postsynaptic dendritic spine per a SP+ or IB4+ boutons was also significantly higher in the CFA-group than control. Number of docked synaptic vesicles attaching to or near the presynaptic membrane at the active zone in the SP+ or IB4+ boutons was significanlty higher in the CFA-group than control. These findings suggest that nociceptive information conveyed via SP+, CGRP+ and IB4+ afferents is processed in a distinct manner, respectively, in the MDH and these boutons show structural platicity at synapse under pathologic pain condition.

Disclosures: Y. Cho: None. **J. Lee:** None. **H. han:** None. **D. Ahn:** None. **H. ko:** None. **Y. Bae:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.04/H24

Topic: D.03. Somatosensation – Pain

Support: NRF 2015R1C1A1A01053484
NRF 2017R1A2B3005753

Yonsei University College of Dentistry Fund (6-2014-0112)

Title: The role of TRPV1 in trigeminal ganglion and brain stem following dental pulp inflammation in rats

Authors: *M. CHA[1], I. SALLEM[2], I.-Y. JUNG[2], B. LEE[1];
[1]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Dept. of Conservative Dent. and Oral Sci. Res. Ctr., Yonsei Univ. Col. of Dent., Seoul, Korea, Republic of

Abstract: Pulpitis produces significant changes in the peripheral nervous system, which induce hyperalgesia. However, the neuronal activity and TRPV1 expression following pulpal noxious stimulation have not yet been investigated in the central nervous system (CNS). The aim of the present study was to verify whether experimentally induced pulpitis activates the expression of TRPV1 and c-Fos, both peripherally and centrally. Acute pulpitis was induced in Sprague-Dawley rats via pulp exposure and application of complete Freund's adjuvant (CFA) (n=13). Saline-treated (n=13) rats and rats that did not undergo tooth preparation (n=13) served as control groups. Three days post-CFA or -saline application, face grooming activity was recorded, and the rats were then euthanized to allow for immunohistochemical analysis of the trigeminal ganglion (TG) and spinal trigeminal nucleus. We found significantly increased pain-like behavior and histological evidence of severe pulp inflammation in CFA-treated animals. C-Fos labelling and TRPV1 immunoreactivity in the TG were significantly higher (both $p<0.05$) in the CFA group than in the control groups. In the spinal trigeminal nucleus, the immunoreactivity for c-Fos was absent in the intermediate region (trigeminal subnucleus interpolaris) in all animals, with comparable expression of TRPV1 among all groups. In contrast, neurons in the trigeminal subnucleus caudalis (TSC) exhibited significant c-Fos immunoreactivity in the CFA group. The expression of TRPV1 did not differ among the three groups, but the superficial laminae of the TSC exhibited significantly higher expression of TRPV1 than did the deep layers. These findings indicated that the TRPV1 channel was significantly involved in nociceptive signal processing in the peripheral nervous system, but not in the CNS, following acute pulp inflammation. Because pulpitis induced some neuronal activation at the brainstem levels, further studies are needed to identify additional transducers that mediate signal transmission from pulpal afferents to their central targets. This research was supported by the Basic Research Program through the National Research Foundation (NRF) funded by the Ministry of Science and ICT (2015R1C1A1A01053484 and 2017R1A2B3005753) and the Yonsei University College of Dentistry Fund (6-2014-0112).

Disclosures: **M. Cha:** None. **I. Sallem:** None. **I. Jung:** None. **B. Lee:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.05/H25

Topic: D.03. Somatosensation – Pain

Support: NIH DC011579

Title: Heterogeneous types of thermosensitive neurons mediate oral temperature coding in the mouse trigeminothalamic tract

Authors: *J. LI, C. LEMON;
Biol., Univ. of Oklahoma, Norman, OK

Abstract: Spinal trigeminal subnucleus caudalis (Vc) neurons projecting to the contralateral thalamus (i.e., trigeminothalamic tract cells) are implicated to mediate sensory-discriminative features of somatosensation. The cell types and coding mechanisms of the oral sensory trigeminothalamic pathway are poorly characterized. Here, we examined oral somatosensory coding by trigeminothalamic tract (VcTT) neurons using extracellular recordings in anesthetized C57BL/6J mice under oral adaptation to 35°C. Orally-delivered stimuli included multiple temperatures (oral ~6°, 14°, 21°, 28°, 35°, 48° and 55°C) and chemesthetic stimuli associated with activation of TRP channels on peripheral trigeminal fibers including 1.28 mM menthol (cooling agent, agonist of TRPM8), 1 mM mustard oil (TRPA1) and 1mM capsaicin (TRPV1). Cells recorded in the Vc were classified as VcTT neurons when they met standard criteria for antidromic activation (stable latency; ability to follow high frequency train; collision test) during weak electrical stimulation of the contralateral thalamus and when post-mortem histological reconstruction of the stimulating electrode tip verified its placement in the contralateral ventroposterior medial thalamus (VPM). Thirty-three thermal-sensitive Vc neurons were identified as VcTT cells. Thus far, two VcTT neurons displayed sensitivity to noxious cold temperatures (~6° and 14°C), noxious hot temperatures (48° and 55°C), and capsaicin. The other 31 VcTT neurons showed responses to cooling temperatures less than 35°C and fired to oral presence of menthol. Cluster analysis revealed VcTT neurons composed 3 groups based on their firing rates to 7 temperatures, with neurons displaying varying selectivity to innocuous cooling (21° and 28°C, group 1, n = 18), cells strongly responsive to extreme cold temperatures (~6° and 14°C; group 2, n = 13), or neurons with broad responsiveness to noxious hot and noxious cold (group 3, n = 2). Cooling- and cold-sensitive VcTT neurons in groups 1 and 2 fired to oral delivery of menthol but not mustard oil, implicating oral cooling activity in these neurons was contributed by input from TRPM8-positive fibers. Multidimensional scaling analyses of neural coding suggested activity across neural groups, as opposed to the activity of any single group, could allow VcTT neurons to systematically signal differences among temperatures, implying

oral thermal processing along the trigeminothalamic tract involves a combinatorial mechanism. Our data indicate that heterogeneous types of VcTT neurons work together to represent thermal stimuli applied to the mouth.

Disclosures: J. Li: None. **C. Lemon:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.06/H26

Topic: D.03. Somatosensation – Pain

Support: NIH Grant DE026499

Title: Estrogen-dependent glia activation in a model for TMJ nociception

Authors: J. K. OLSON, M. M. RAHMAN, R. THOMPSON, *D. A. BEREITER;
Diagnos. and Biol. Sci., Univ. of Minnesota Sch. of Dent., Minneapolis, MN

Abstract: Temporomandibular joint disorder (TMD) is the most common non-dental orofacial pain condition. TMD is most prevalent in women and presents with no overt signs of injury or inflammation to the joint. Neuron-glia interactions have been suggested to play an important role in pain conditions through neuroimmune mechanisms. Activation of satellite glia cells in the periphery and microglia in the central nervous system have been demonstrated in other pain conditions. The goal of this project was to determine the role of neuroimmune mechanisms in TMD especially as related to the prevalence in female patients. To address the issue of estrogen status on neuroimmune relations male, ovariectomized (OvX) female and OvX rats treated estradiol (OvXE, 30μg/kg, sc) were injected with a non-tissue damaging dose (10μg) of Complete Freund's Adjuvant (CFA) into the TMJ. Evan's Blue dye recovery indicated a transient increase in vascular permeability that returned to baseline by 10 days post CFA. Trigeminal ganglia (TG) had infiltration of immune cells 4 days after CFA, especially in male rats, but decreased by 10 days. Satellite glia in the TG were activated at 4 days and remained activated at 10 days post CFA. Satellite glia activation was associated with increased expression of pro-inflammatory cytokines, especially IL-1β, with highest levels in OvXE rats. Trigeminal brainstem (Vc) samples displayed no evidence of infiltration of immune cells at 4 days or at 10 days post CFA. Microglia were activated at 4 days and remained activated at 10 days post CFA, predominantly in OvXE rats. Significantly, microglia expressed high levels of pro-inflammatory cytokine, IL-1β, in OvXE compared to male rats that increased with time post CFA injection. Since IL-1β and NLRP3 inflammasomes are associated with purinergic receptor, P2X7, it was notable that P2X7R expression increased preferentially in OvXE rats after CFA injection. To determine if TMJ inflammation leads to persistent enhanced nociceptive behavior, masseter

muscle EMG activity (MMemg) was evoked by intra-TMJ injections of ATP (0.01-1mM) in 10d CFA rats. All groups displayed enhanced MMemg activity after CFA which was higher in OvXE compared to male rats. These data indicate that transient TMJ inflammation is sufficient to cause persistent increases in the activation of satellite glia in TG and of microglia in Vc in an estrogen-dependent manner. These data support the hypothesis that chronic TMD is an inflammatory pain condition, predominantly in females, that is maintained by persistent increases in innate immune activation at multiple sites within trigeminal pain pathways.

Disclosures: J.K. Olson: None. **M.M. Rahman:** None. **R. Thompson:** None. **D.A. Bereiter:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.07/H27

Topic: D.03. Somatosensation – Pain

Support: NIH grants DE018661
NIH grants DE023090

Title: A role of voltage-gated potassium channel K_v4.3 in controlling orofacial nociception of cooling temperatures

Authors: *Y. T. CHANG[1], H. KANDA[2], J. G. GU[1];
[1]Anesthesiol. and Perioperative Med., UAB, Birmingham, AL; [2]Pharmacol., Hyogo Univ. of Hlth. Sci., Hyogo, Japan

Abstract: Potassium channels such as A-type voltage-gated potassium channels play important roles in controlling neuronal excitability and nociception in the somatosensory system. K_v4.3 channels, a subtype of A-type voltage-gated potassium channels, have been shown to be expressed in nociceptive somatosensory neurons in the dorsal root ganglions (DRG) and trigeminal ganglions (TG). In the present study, we first determined whether Kv4.3 channels significantly contribute to voltage-activated outward K^+ currents in small-sized nociceptive-like TG neurons of V2 regions (V2 TG) using the patch-camp recording technique. The patch-clamp recordings were performed on the V2 TG neurons *in situ* in an *ex vivo* TG preparations. We found that the voltage-activated outward K^+ currents in many of the V2 TG neurons could be inhibited by the K_v4.3 channel blocker phrixotoxin-2 (200 nM), suggesting the presence of functional K_v4.3 channels in these V2 TG neurons. We further determined whether K_v4.3 channels are involved in controlling orofacial nociception and whether inhibition of these channels can result in orofacial cold hyperalgesia. To address these issues, we performed the orofacial operant test and determined the effect of phrixotoxin-2 on the orofacial operant test.

We found that intracutaneous injection of 0.8 mg phrixotoxin-2 significantly reduced orofacial operant behaviors at the cooling temperature of 5 ^{o}C, suggesting the induction of orofacial cold hyperalgesia following the inhibition of K_v4.3 channels at the peripheral nerve endings. Collectively, K_v4.3 may play an important role in controlling orofacial nociception of cooling temperatures and compromising their functions may lead to cold hyperalgesia.

Disclosures: **Y.T. Chang:** None. **H. Kanda:** None. **J.G. Gu:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.08/H28

Topic: D.03. Somatosensation – Pain

Support: NIH grant DE018661
NIH grant DE023090

Title: Effects of cooling temperatures on the excitability of nociceptive-like trigeminal ganglion neurons that innervate the orofacial skin of rats

Authors: *Y. OKUTSU[1], J. GU[2];
[1]Univ. of Alabama At Birmingham, BIRMINGHAM, AL; [2]Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Pain sensations can be affected by temperatures; for example, pathological pain can be acerbated at cooling temperatures. We have previously reported that the excitability of many trigeminal ganglion neurons (TGs) became increased at cooling temperatures (Kanda et al. 2017). In the present study, we determined whether nociceptive-like TG neurons that innervate the skin of orofacial regions also become more excitable. Sprague-Dawley rats aged 7-10 weeks were used and the fluorescent dye DiI was intracutaneously injected into the orofacial areas of the rats. Five to seven days after DiI injections TGs were dissected out from the rats and patch-clamp recordings were applied to the DiI-labeled small-sized TG neurons (20 to 34 μm in diameters) situated in the whole-mount TG preparations. These small-sized DiI-labeled TG neurons usually had shoulders in the repolarization phase of their action potentials (AP) and their action potential widths were > 2.0 ms when recorded at 24^{o}C, suggesting these neurons are mainly C-fiber nociceptors. The rheobase for action potential firing in these TG neurons were found to be significantly lower at 15^{o}C than at 24^{o}C, suggesting an enhanced excitability at cooling temperatures. Membrane input resistances and action potential widths of these neurons were significantly increased at cooling temperature of 15^{o}C. Under the voltage-clamp mode, both inward currents and outward currents elicited by voltage steps were suppressed at 15^{o}C. Further studies are needed to determine ion channels responsible for the changes of the excitability and

other electrophysiological properties of these nociceptive-like TG neurons at cooling temperatures. The study was supported by NIH grants DE018661 and DE023090 to J.G.G.

Disclosures: **Y. Okutsu:** A. Employment/Salary (full or part-time):; University of Alabama At Birmingham. 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 grants DE018661 and DE023090 to J.G.G. **J. Gu:** A. Employment/Salary (full or part-time):; University of Alabama At Birmingham. 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 grants DE018661 and DE023090 to J.G.G.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.09/H29

Topic: D.03. Somatosensation – Pain

Support: KAKEN

Title: Potential role of TRPV1 antagonism for relieving orthodontic force induced pain

Authors: ***K. ADACHI**[1], N. HASEGAWA[2], T. TSUCHIYA[2], M. YUGAWA[2], A. SASAKI[2], N. SUDA[2];
[1]Pharmacol., [2]Orthodontology, Meikai Univ. Sch. of Dent., Sakado, Japan

Abstract: The orthodontic treatment is common therapy to improve functional and aesthetic dental issues , but many patients complain about pain during tooth movement. We have reported that the jaw-opening reflex (JOR) excitability is increased in 1 (D1)-3 (D3) days and is decreased in 7 days (D7) after orthodontic force application in rats. In this model, generally anesthetized rats were applied continuous orthodontic force by Ni-Ti coil spring to only right maxillary first molar. Because the period of temporal alteration of the JOR excitability is similar with that of orthodontic pain in clinic, this animal model may allow us to investigate the novel approach to treatment of orthodontic pain. Consistent with the hypothesis, one day application of Transient Receptor Potential Vanilloid 1 (TRPV1) antagonists (A-889425: 5-10 μmol/kg, AMG9810: 10-15 μmol/kg) and aspirin (560 μmol/kg) significantly reduced JOR excitability, and prolonged application of minimum analgesic dose of these chemicals did not alter JOR excitability at D7. In this animal model, body weight was significantly reduced at D1 and D3, then it was gained to pre-operation level. However, significant weight loss was continuously observed until D7 in aspirin, not TRPV1 antagonists, treated animals. Although these analgesic chemicals reduced the

expression of mature osteoclasts at D3 as insignificantly and D7 as significantly, the distance of tooth movement was not altered. These results emphasized the potential role of TRPV1 antagonism for relieving orthodontic force-induced pain. In addition, TRPV1 antagonists significantly reduced tooth movement related pain at D1, the period without mature osteoclasts expression, suggests TRPV1 antagonism may alter other biological features and we added the investigation for alteration of inflammatory cytokines by application of minimum analgesic does of these chemicals by antibody arrays. After evaluation of JOR excitability at D1, the right maxillary first molar was extracted and processed with common procedure to obtain sampling solution. In comparison between intact animal, the significant increase of CINC2, CINC3, IL-6, TIMP1 and TNF was induced by orthodontic force application. Aspirin treatment significantly reduced the expression of IL-6 only. On the other hand, both TRPV1 antagonists significantly reduced the expression of CINC2, CINC3 in addition to IL-6. Taken together, reduction of wider inflammatory cytokines by TRPV1 antagonism may relate with the lower analgesic dose of antagonists and analgesic effects at non-acidic environment.

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Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.10/H30

Topic: D.03. Somatosensation – Pain

Support: NIH DE023846
NIH DE027731

Title: Nociceptors expressing TRPV1 and trigeminal nucleus neurons expressing NK1 mediate orthodontic pain

Authors: *S. WANG[1], M. KIM[1], K. ONG[1], E.-K. PAE[2], M.-K. CHUNG[1];
[1]Neural and Pain Sci., [2]Dept. of Orthodontic and Pediatric Dent., Univ. of Maryland, Baltimore, MD

Abstract: Orthodontic force produces mechanical irritation and inflammation in periodontium, which inevitably accompanies pain. Despite its high prevalence, treatment of orthodontic pain is not effective. Determining detailed neural mechanisms involving peripheral and central nervous system should be critical to improve the management of orthodontic pain. Periodontal ligament is projected by peptidergic nociceptors, which is enriched with transient receptor potential vanilloid 1 (TRPV1), a receptor for capsaicin. Trigeminal subnucleus caudalis (Vc), is critical for relaying orofacial nociceptive signal into brain. A group of second-order neurons in the

superficial dorsal horn of Vc express neurokinin 1 receptor (NK1), a receptor for substance P, and receive inputs from peptidergic nociceptors. However, the contribution of these nociceptive neurons to orthodontic pain has not been determined. Orthodontic force of 10g produced reliable tooth movement in mice. Orthodontic pain was evaluated by measuring mouse grimace scale (MGS) and bite force (BF), which could represent spontaneous pain and chewing-evoked pain, respectively. Orthodontic force increased MGS and decreased BF, which peaked at 1d and returned near to sham level at 7d. Using targeted chemical ablation of specific subsets of neurons, we determined the contribution of TRPV1+ nociceptors and NK1+ Vc neurons to orthodontic pain behaviors in mice. Ablation of TRPV1+ nociceptors by injecting resiniferatoxin into trigeminal ganglia significantly attenuated orthodontic force assessed by MGS and BF. Chemical ablation of NK1+ Vc neurons by injecting saporin conjugated with substance P into Vc also significantly reduced the extent of changes in MGS and BF by orthodontic force. These results suggest that TRPV1+ trigeminal nociceptors and NK1+ Vc neurons constitute a major neural pathway for transmission of orthodontic pain, which is a fundamental neural mechanism of orthodontic pain transmission. The new mouse model of orthodontic pain will be useful for mechanistic study to develop novel approaches for painless orthodontics.

Disclosures: **S. Wang:** None. **M. Kim:** None. **K. Ong:** None. **E. Pae:** None. **M. Chung:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.11/H31

Topic: D.03. Somatosensation – Pain

Support: Palliative Care Pilot Award by the American Cancer Society
Pilot project award by the Institute of Integration of Medicine and Science
NIH R01DE027223

Title: Sensory neuronal subtypes innervating mouse tongue

Authors: *D. ARRIS[1], P. WU[1], M. GRAYSON[1], C.-N. HUNG[2], S. RUPAREL[1];
[1]Endodontics, [2]Mol. Med., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: The current study identified and characterized lingual sensory neurons based on a neuronal subtype classification previously characterized in the dorsal root ganglion (DRG) neurons. We employed immunohistochemistry on transgenic reporter mouse lines as well as single-cell PCR of known markers of neuronal subtypes to characterize neuronal subtypes innervating the tongue. Markers expressed in retrogradely labeled TG neurons were evaluated for the proportion of neurons expressing each marker, intensity of expression, and overlapping genes. We found that tongue-innervating sensory neurons primarily expressed CGRP, TRPV1,

TrkC, 5HT3A and Parvalbumin. These markers correspond to peptidergic and a subgroup of non-peptidergic C-nociceptors, peptidergic A nociceptors, proprioceptors and myelinated low-threshold mechanoreceptors (LTMRs). Interestingly, as reported previously, we also found several differences between TG and DRG neurons. Our expression data also correlated with functional subtypes as observed with single-fiber recordings of lingual afferents.

Disclosures: D. Arris: None. **S. Ruparel:** None. **M. Grayson:** None. **P. Wu:** None. **C. Hung:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.12/H32

Topic: D.03. Somatosensation – Pain

Support: NIH R01

Title: Vascular action in a mouse model of migraine

Authors: *L. K. BALCZIAK[1], A.-S. WATTIEZ[1], B. N. MASON[1], M. W. CHAPLEAU[1,2], A. F. RUSSO[1,3];
[1]Mol. Physiol. and Biophysics, [2]Intrnl. Med., [3]Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Migraines are the leading cause of disability in the US and affect around 700 million people worldwide. Symptoms include pain, photophobia, phonophobia, nausea, and vomiting. The neuropeptide calcitonin gene-related peptide (CGRP) is a key mediator of migraine and drugs that block CGRP action are now being used to prevent migraine. However, the mechanism by which CGRP causes migraine remains unknown. Since CGRP is a potent vasodilator, we reasoned that this activity may contribute to migraine. The vasculature in the meninges is innervated extensively by trigeminal nociceptive fibers that express CGRP and mechanosensitive fibers that express the CGRP receptor. Changes to the vasculature may increase firing of these neurons, leading to peripheral sensitization and ultimately to migraine symptoms. We have tested this hypothesis by manipulating the systemic blood pressure in mice and measuring light aversion and cutaneous allodynia in mice. These assays serve as surrogates for photophobia and touch sensitivity that occurs during migraine attacks. Specifically, we have tested the actions of caffeine. Caffeine is the most common psychoactive and vasoactive drug in the world and is often used in conjunction with anti-inflammatory agents to treat migraine. Our preliminary data indicate that caffeine and other vasoconstrictor agents can prevent CGRP-induced vasodilation and partially alleviate CGRP-induced light aversion and allodynia. The importance of the vasculature was further supported by enhanced CGRP-induced light aversion in transgenic mice that have elevated CGRP receptors in vascular smooth muscle. Current studies are measuring

dilation of the middle meningeal artery following treatments in awake mice by multiphoton imaging. These findings lay the foundation for preclinical testing of combinatorial treatments of caffeine and the new CGRP drugs to alleviate migraine symptoms.

Disclosures: L.K. Balcziak: None. **A. Wattiez:** None. **B.N. Mason:** None. **M.W. Chapleau:** None. **A.F. Russo:** F. Consulting Fees (e.g., advisory boards); Lily, Alder, Amgen, Novartis.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.13/H33

Topic: D.03. Somatosensation – Pain

Support: NIH DE025970

Title: Fluctuating estrogen levels exacerbate serotonin-evoked pain signaling in trigeminal sensory neurons

Authors: *S. LULLA, H. R. MCDONALD, D. L. AVERITT;
Biol., Texas Woman's Univ., Denton, TX

Abstract: Craniofacial pain disorders involving trigeminal sensory neurons disproportionately affect women. These neurons express transient receptor potential vanilloid 1 ion channel (TRPV1). TRPV1, activated by capsaicin and heat, can be sensitized by inflammatory mediators causing increased calcium influx and calcitonin gene-related peptide (CGRP) release leading to peripheral sensitization. In the peripheral nervous system, serotonin (5HT) is released and acts as a proinflammatory and pronociceptive mediator. We have shown that 5HT hindpaw injection evokes higher thermal hyperalgesia and mechanical allodynia in proestrus and estrus female rats, when gonadal hormones fluctuate. Since craniofacial pain disorders are also linked to menstrual cycle stages, gonadal hormones may modulate trigeminal pain. However, estrogen (E2) effects are controversial as E2 can exacerbate trigeminal pain at high or low levels. We hypothesized that *fluctuating levels of E2 exacerbate 5HT-evoked pain and E2 enhances capsaicin-evoked pain signaling in the presence of 5HT in trigeminal sensory neurons.* Adult male, cycling, and ovariectomized (OVX) female rats received a vibrissal pad (VP) injection of 1.5 µg or 3 µg 5HT, combined with 1 µg capsaicin, or vehicle (50µl). Nocifensive behavior was counted as number of forelimb swipes over the injected area. A separate group of OVX rats received capsule implants of 5% E2, 10% E2, or vehicle and another group received an acute injection of 2 µg E2, 20 µg E2, or vehicle before 5HT-evoked nocifensive behavior testing. Trigeminal ganglia were extracted from OVX rats and cultured for 5 days. Neurons were treated with HBSS buffer, E2 receptor agonists, or vehicle followed by 5HT+capsaicin stimulation. Superfusate was collected after each 15-min treatment and quantified for CGRP release or calcium influx was recorded

during each treatment. Data was analyzed by 2-way ANOVA and individual groups were compared by Tukey's posthoc analysis. We report that 3 µg 5HT evoked higher nocifensive behaviors in estrus and proestrus females. With capsaicin, 3µg 5HT evoked nocifensive behaviors in estrus females and males, whereas, 1.5 µg 5HT evoked nocifensive behaviors in proestrus females. In OVX rats, 2 µg E2 exacerbated and 20 µg E2 slowed the onset of nocifensive behaviors, whereas, no difference was seen in capsule treated group. E2 also enhanced 5HT-potentiated CGRP release from cultured trigeminal neurons and this effect does not appear to occur via membrane-bound estrogen receptor. Our data indicates that estrogen concentration is an important factor in modulating the pronociceptive effects of 5HT on trigeminal sensory neurons.

Disclosures: S. Lulla: None. **H.R. McDonald:** None. **D.L. Averitt:** None.

Poster

052. Somatosensation: Trigeminal Pain Circuits and Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 052.14/H34

Topic: D.03. Somatosensation – Pain

Support: NIH Grant DE025970

Title: Gonadal hormones modulate peripheral serotonin levels and plasticity in serotonin receptor expression: A potential mechanism underlying sex differences in trigeminal pain

Authors: *T. M. HICKMAN, S. LULLA, H. R. MCDONALD, E. MONTELONGO, R. HORNUNG, D. L. AVERITT;
Biol., Texas Woman's Univ., Denton, TX

Abstract: Sex differences in the immune system are being increasingly reported and may play a role in pain conditions that are more prevalent in women. The neurotransmitter serotonin (5HT) can be sequestered and/or synthesized to be released by immune cells, macrophages, mast cells, and injured epithelial cells in areas of tissue damage. 5HT then initiates or sensitizes pain signaling by acting on excitatory 5HT receptors (such as $5HT_{2A}$ and $5HT_{3A}$) expressed on nociceptors. Given the dual role of 5HT in the immune system and nociception, it is possible that sex differences in 5HT release and/or activity at nociceptors may be one factor underlying the greater prevalence of some pain conditions in women. Further, this system may be modulated by gonadal hormones. We have recently linked fluctuating estrogen (E2) levels to enhanced rodent pain behaviors and others have shown that E2 can upregulate 5HT synthesizing enzymes in rodent sensory neurons. Currently, there are no reports of E2's role on 5HT release and literature on 5HT receptor fluctuation across the estrous cycle of rodents is controversial. No study has yet analyzed 5HT receptor expression in human sensory neurons. Here we hypothesized that E2

upregulates 5HT release during inflammation in rodents and increases excitatory 5HT receptor expression in human sensory neurons. Adult male, cycling, and ovariectomized (OVX) female rats received a left vibrissal pad (VP) injection of complete Freund's adjuvant (CFA) and right VP injection of saline. Interstitial fluid was collected from tissue biopsies 24-hours post-inflammation. Trunk blood was collected from a separate group of rats that received intraplantar hindpaw injection of CFA for 24 hours. We also obtained supernatant from virus-infected macrophage samples. 5HT release was quantified using ELISA. We report that cycling females have significantly higher 5HT content in the saline-injected, but not CFA-injected, VP as compared to males and OVX females. The trunk blood of diestrus females had higher 5HT as compared to proestrus and estrus females. Further 5HT was detected in the macrophage supernatant. Since estrogen plays a major role in gene regulation, we next assessed $5HT_{2A}$ and $5HT_{3A}$ receptor expression in trigeminal nerve-innervated human tooth pulp collected from females across the menstrual cycle. Our preliminary data indicates that $5HT_{3A}$, but not $5HT_{2A}$, receptor protein expression may be upregulated during the luteal phase. Overall, our date indicate that serotonergic activity in the periphery may be sexually dimorphic and warrants further investigation into the link between estrogen and immune-mediated 5HT.

Disclosures: T.M. Hickman: None. **S. Lulla:** None. **H.R. McDonald:** None. **E. Montelongo:** None. **R. Hornung:** None. **D.L. Averitt:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.01/H35

Topic: D.03. Somatosensation – Pain

Support: ERT

Title: Value of patient training on reporting rescue medication use: Relevance to clinical trials in migraine

Authors: *K. M. DUMAIS, S. M. DALLABRIDA;
Clin. Sci. and Consulting, ERT, Boston, MA

Abstract: Self-reported rescue medication use is frequently used to support drug efficacy in clinical trials, yet the concept of rescue medication may not be well understood by patients. This is especially critical for studies involving pain, such as migraine, where medication use is required to define primary endpoint (i.e., migraine days) or eligibility. Participants from the general public (N=934) were surveyed online regarding their understanding of rescue medication. Participants were then given a 1-sentence written instruction on the meaning of rescue medication to examine whether this would improve understanding. Demographic data and

existence of migraines were also collected. Prior to reading the 1-sentence instruction, only 22% of participants correctly identified rescue medication as any medication taken to treat your pain other than the investigational medication. About a third of participants (32%) thought rescue medication was a medication used to rescue you from a dangerous situation. A similar low correct response rate was found in those who experience migraines (21%), had previously participated in a clinical trial (26%), or who have a college education or higher (29%). After the 1-sentence instruction, 435 participants in total changed from an incorrect response to a correct response, increasing the percentage of participants who correctly defined rescue medication to 66% ($p<0.0001$, McNemar's Test). A one sentence training significantly increased the number of participants correctly defining rescue medication in all subgroups analyzed (experience migraines [68%], participated in a clinical trial [70%], college education or higher [80%], all p's < 0.0001, McNemar's Test). Accurate reporting of rescue medication by clinical trial subjects is imperative for identifying investigational drug efficacy and reducing noise and false data. Most participants surveyed, even those who experience migraines, have participated in clinical trials, or have advanced degrees, did not understand the meaning of the term rescue medication, and a simple 1-sentence instruction significantly increased participants' understanding. We expect that a more comprehensive training developed for a clinical trial including more detailed descriptions and scenario based explanations could increase patients' understanding beyond what is reported here. These findings should encourage the use of subject training in clinical trials in order to optimize patients' understanding and increase accurate self-reporting of rescue medication.

Disclosures: **K.M. Dumais:** A. Employment/Salary (full or part-time):; Authors are employees of ERT, who funded this research. **S.M. Dallabrida:** A. Employment/Salary (full or part-time):; Authors are employees of ERT, who funded this research..

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.02/H36

Topic: D.03. Somatosensation – Pain

Support: NIH NINDS R43-NS108824-01
NIH NINDS NS-019108
NCATS UL1 TR000430
the George Schultz Innovation Fund from the Polsky Center for Entrepreneurship and Innovation at the University of Chicago
Sponsored Research Agreement from Seurat Therapeutics, Inc.

Title: Nose-to-brain insulin-like growth factor-1 abrogates trigeminal system activation from spreading depression

Authors: L. A. WON[1], *R. P. KRAIG[2];
[1]Neurol., Univ. Chicago, Chicago, IL; [2]Neurol., Univ. of Chicago, Chicago, IL

Abstract: Migraine is a health care burden in need of improved therapeutics. This void has begun to be filled by focus on calcitonin gene related peptide (CGRP), an inflammatory molecule released from the trigeminal system (TS) with migraine. New anti-CGRP drugs show exciting promise. These agents are antibodies designed to inhibit or absorb circulating CGRP to block nociceptive activation of the TS. As such, they do not influence the cause [i.e., underlying brain hyperexcitability (HE) and TS over activity] of migraine, but instead influence the consequence (i.e., CGRP release) which might account for their partial impact on migraine. We have taken a different approach. Increased exercise and creative thought (termed environmental enrichment or EE) is known to protect brain against subsequent neurological disease. EE occurs with a rise in insulin-like growth factor-1 (IGF-1) which is neuroprotective. We showed that IGF-1 reduces brain HE associated with migraine modeled using spreading depression (SD) in brain slice cultures. Furthermore, nose-to-brain (N2B) delivery of IGF-1 protects against SD in whole animals. Here we extend that work to determine the impact of N2B IGF-1 pretreatment a day before recurrent SD on TS activation. N2B IGF-1 significantly reduced ($p<0.001$) SD-induced trigeminal ganglion oxidative stress [(OS), measured via malondialdehyde immunostaining] and immunostaining for CGRP by 82% and 44%, respectively. In fact, IGF-1 reduced CGRP in the trigeminal ganglion of naïve animals (without SD activation) by 75% compared to vehicle. Thus, N2B IGF-1 treatment does not appear to induce headache, a concern from systemic use of IGF-1. Also, N2B IGF-1 showed no evidence of hypoglycemia. Finally N2B administration of IGF-1 significantly ($p=0.003$) reduced caudal trigeminal nucleus activation (i.e., c-fos immunostaining). These results show for the first time that N2B IGF-1 is an effective means to abrogate TS activation associated with migraine (modeled using SD). Others have shown that the highest levels of IGF-1 occur in the trigeminal ganglion after N2B IGF-1 in rodents. Thus, this delivery method may be well-suited for translation to the human condition. Furthermore, while specific mechanisms for the protective impact of N2B IGF-1 treatment for migraine remain to be determined, our data here and prior work from our lab strongly support blocking OS as a key mechanism. Perhaps N2B IGF-1 will mitigate the depletion of antioxidants seen in migraine patients that leads to worsening of this disorder.

Disclosures: L.A. Won: None. **R.P. Kraig:** A. Employment/Salary (full or part-time):; University of Chicago, Chicago, IL. 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.; Sponsored Research Agreement from Seurat Therapeutics, Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH NINDS R43-NS108824-01 and NS-019108, NCATS UL1 TR000430, the George Schultz Innovation Fund from the Polsky Center for Entrepreneurship and Innovation at the University of Chicago. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-Founder and CSO Seurat Therapeutics, Inc. F. Consulting Fees (e.g., advisory boards); CVS Caremark consultant. Other; none.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.03/H37

Topic: D.03. Somatosensation – Pain

Title: a9a10 nicotinic acetylcholine receptor antagonist as a potential target for acute migraine therapy

Authors: *M. M. SHAH[1], J. SANZ[1], J. MCINTOSH[3], F. PORRECA[4], J. Y. XIE[2];
[2]Dept. of Basic Sci., [1]NYIT Col. of Osteo. Med. at Arkansas State Univ., Jonesboro, AR; [3]Univ. of Utah, Salt Lake Cty, UT; [4]Dept Pharmacol., Univ. of Arizona Col. of Pharm., Tucson, AZ

Abstract: Migraine is a disabling disorder affecting 11-13% of the adult population in the United States. Existing acute treatments provide satisfactory pain relief to less than 50% of migraine sufferers. Additionally, if overused, current acute migraine medications are associated with a risk of developing medication overuse headache (MOH), a form of chronic migraine that is even more intractable to treatments. The autonomic nervous system has long been implicated in migraine pathophysiology, as activation of the α9α10 nicotinic acetylcholine receptor (nAChR) might sensitize nociceptive afferents following release of CGRP and inflammatory cytokines. Therefore, α9α10 nAChR antagonists may represent a viable novel mechanism for acute treatment of migraine-related pain. RgIA4 is a novel peptidic α9α10 nAChR antagonist that has high potency and selectivity to both rodent and human α9α10 nAChRs. We hypothesized that RgIA4 might be efficacious in alleviating migraine-related pain in a dose- and time-dependent manner and with diminished possibility of development of MOH due to the peripheral restriction of its target and antagonist mechanism. We developed a model of medication overuse headache induced by exposure of rats to sumatriptan to induce vulnerability to presumed migraine triggers including stress. Adult, male Sprague-Dawley rats were primed with sumatriptan (0.6 mg/kg/day) over 7 days by continuous subcutaneous infusion via osmotic minipump. Sumatriptan treatment resulted in periorbital and hindpaw allodynia that resolved within 2 weeks following discontinuation of the drug. Animals were then exposed to two episodes of bright light stress (BLS, 3300 lux) for 1h/day on two consecutive days resulting in precipitation of wide-spread cutaneous allodynia in rats previously receiving sumatriptan. Allodynia was delayed and generalized and peaked 2-3h after the 2nd BLS. Administration of RgIA4 (100 nmol/rat, i.m.) at 2 hours post-BLS reversed the periorbital and hindpaw allodynia ($P<0.05$ compared to pre-dose baseline). Our results support the hypothesis that the α9α10 nAChR antagonist RgIA4 alleviates stress-induced cephalic and extracephalic allodynia in a model of medication overuse headache in rats. The data suggest that α9α10 nAChR antagonists may represent a viable mechanism for acute treatment of migraine-related pains.

Disclosures: **M.M. Shah:** None. **J. Sanz:** None. **J. McIntosh:** None. **F. Porreca:** None. **J.Y. Xie:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.04/H38

Topic: D.03. Somatosensation – Pain

Support: Centre for Neuor Skills-Clinical Research and Education Foundation

Title: Altered functional connectivity during a spontaneous visual aura in a migraine patient

Authors: *J. ASHLEY[1], M. ASHLEY[2,3], G. S. GRIESBACH[4], C. K. SINGH[5], N. G. HARRIS[6]; [1]Ctr. for Neuro Skills Clin. Res. and Educaiton Fndn., Bakersfield, CA; [2]Ctr. for Neuro Skills, Bakersfield, CA; [3]Neurol., Univ. of California Los Angeles, Los Angeles, CA; [4]Clin. Res., Ctr. For Neuro Skills, Encino, CA; [5]Ctr. For Neuro Skills, Bakersfield, CA; [6]Neurosurg., Dept. Neurosurgery, UCLA, Los Angeles, CA

Abstract: We report on MRI data from a subject who experienced a spontaneous visual aura during resting state functional magnetic resonance imaging (rsfMRI). Data was acquired immediately prior to onset of migraine with aphasia and hemiplegia. The mechanisms underlying visual aura are intricate and incompletely understood. Capture of this occurrence during rsfMRI may inform the understanding of pathophysiology, and may thus have implications for management of migraine. The advent of new technologies such as rsfMRI carries the potential for increasing our understanding of this phenomena**.** In this instance, the subject was able to verbalize onset of the visual aura, described as a flash of light. The subject had two prior rsfMRI sessions completed 6 and 7 months prior, when no such aura event occurred. The 3 sets of data were processed for functional connectivity using correlational analysis and the prior sets were used for comparison. Visual examination of the voxel-based time-courses indicated significantly increased signal r values in the posterior brain regions, especially in the visual cortex, when compared to the anterior regions and to all regions within the two other data sets. Seed-based connectivity analysis from the left and right visual cortex indicated greater connectivity and over a larger connected region from the visual cortex when compared to the previous two datasets. The capture of this spontaneous visual aura during resting state fMRI has the potential to provide important information regarding the underlying neural and vascular mechanisms.

Disclosures: **J. Ashley:** A. Employment/Salary (full or part-time):; part-time. **G.S. Griesbach:** A. Employment/Salary (full or part-time):; full time. **C.K. Singh:** A. Employment/Salary (full or part-time):; full time. **N.G. Harris:** None. **M. Ashley:** A. Employment/Salary (full or part-time):; part-time.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.05/H39

Topic: D.03. Somatosensation – Pain

Support: NS040538
NS070711

Title: A bioactive lipid mechanically sensitizes trigeminal neurons: A role in migraine headache

Authors: *F. MOEHRING, C. L. STUCKY;
Cell Biology, Neurobio. & Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Five million people in the US experience monthly migraine attacks. While the exact causes of migraine are unknown, it is hypothesized that trigeminal ganglia (TG) neurons, including those that innervate the dura, exhibit altered chemical and mechanical sensitivity during migraine attacks. To date, no study has compared the mechanical responsiveness of TG to dorsal root ganglia (DRG) somata or examined how neuronal mechanical sensitivity may change in migraine models.
To address this question, the mechanical responsiveness of naïve TG sensory neuron somata was compared to that of DRG neurons using whole cell patch clamp recordings and focal mechanical stimulation. At higher indentations (i.e. > 4.5μm), TG neurons exhibited larger mechanical currents and altered current profiles compared to lumbar DRG neurons.
Because soluble factors in the bloodstream have long been suggested to contribute to migraines, we examined the literature for factors upregulated in the bloodstream of migraineurs and tested the impact of these factors on mechanical sensitivity of TG neurons. A lipidomic study by Ren et al. (2018) recently reported that specific species of the bioactive lipid lysophosphatidylcholine (lysoPC) are dysregulated in the serum of migraine patients. To determine whether lysoPC can directly induce migraine-like behaviors in rodents, we performed dural injections of lysoPC in naïve mice and measured periorbital mechanical sensitivity. Mice injected with lysoPC developed pronounced facial mechanical hypersensitivity that lasted ~48 hrs. TG neurons isolated from mice that received a dural lysoPC injection *in vivo* also exhibited larger mechanically-evoked currents, thus correlating with the behavioral phenotype.
One putative target of lysoPC activity is the Transient Receptor Potential Canonical 5 receptor (TRPC5). To determine the involvement of this ion channel in the lysoPC-induced migraine-like behaviors, we co-injected lysoPC and a TRPC5 antagonist onto the dura of mice; inclusion of the inhibitor protected against lysoPC-induced facial mechanical hypersensitivity. Furthermore, global TRPC5 knockout (KO) mice did not develop facial hypersensitivity following dural lysoPC injections and TG neurons from TRPC5 KO mice injected with lysoPC did not differ in

the current profile or current amplitude. Overall, these data demonstrate that lysoPC can induce migraine-like behaviors in mice and that lysoPC is able to sensitize TG neurons to mechanical force, therefore implicating lysoPC as a soluble factor that may contribute to migraines.

Disclosures: F. Moehring: None. **C.L. Stucky:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.06/H40

Topic: B.04. Ion Channels

Support: NSF (DMS 1516176)
Ronald E. McNair

Title: Eeg brain source analysis of cortical spreading depression propagation

Authors: *D. BORREGO, C. MONCION, J. J. RIERA;
BME, Florida Intl. Univ., Miami, FL

Abstract: Cortical spreading depression (CSD) has been described as a wave of neuronal depolarization that spreads through the cortex at approximately 2-5 mm/min leading to a short period of neuronal activity silencing (Leão, 1944). CSD is considered to be the underlying cause for the sensory disturbances in the migraine aura (Lauritzen, 1994). Electroencephalography (EEG) signatures of the CSD have been difficult to characterize in patients with migraine due to the absence of intracranial DC potential, which is needed to detect the CSD. Therefore, a combination of scalp EEG and intracranial DC potentials from rats has been used to identify these CSD signatures on the scalp (Elmar Busch et al., 1995; Bastany et al., 2016). However, because of the small head size, rat animal models are limited to a few electrodes. In this study, we use a new technology consisting of a 32-electrode high-resolution EEG mini-cap (Fig.1A, Valdes-Hernandez et al., 2016) to characterize the spatiotemporal profiles of scalp EEG while CSDs propagate throughout the cortical sheet. A total of 10 male Wistar rats (age 3-8 weeks) were used. To induce CSDs, we performed a small craniotomy posterior to lambda on the right hemisphere and perfused the brain tissue with a solution of potassium acetate (CH_3CO_2K) via a silicon tube under the EEG mini-cap (Fig.1B). Under this particular preparation, undesirable changes in the volume conducting properties of the rat head are minimized. Specific biomarkers, i.e., propagation velocities and wave-front dynamic deformations, were calculated using the estimated cortical current sources. Identification of scalp EEG biomarkers of CSD through the use of preclinical animal models is critical to create effective therapeutic strategies for the treatment of migraines in humans, the most common neurological disorder affecting more than 37 million people in the US.

Keywords: CORTICAL SPREADING DEPRESSION; MIGRAINE AURA; ELECTROENCEPHALOGRAPHY
Funding: D. Borrego is a R.E. McNair Scholar. NSF (DMS 1516176)
Figure 1
Perfusion Setup
EEG Mini-Cap
B
A
Craniotomy
Electrodes
Silicon Tube
CH_3CO_2K

Disclosures: D. Borrego: None. **C. Moncion:** None. **J.J. Riera:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.07/H41

Topic: D.03. Somatosensation – Pain

Title: Can osteopathic manipulative treatment relieve migraine pain in rats?

Authors: *K. BYRD[1], B. CHUNG[1], K. SHARMA[3], J. XIE[1], R. FLEMING[2];
[1]Dept. of Basic Sci., [2]Dept. of Osteo. Manipulative Med., New York Inst. of Technol. Col. of Osteo. Med. at Arkansas State Univ., Jonesboro, AR; [3]Biol. Sci. and Arkansas Biosci. Inst., Arkansas State Univ., Jonesboro, AR

Abstract: Migraine is a disabling disorder characterized by recurrent unilateral throbbing cephalic pain often associated with hypersensitivity to a variety of external stimuli including light and sound. Initiating events remain unknown, although the ultimate activation of the trigeminovascular system is considered to be essential for pain. Current pharmacologic treatments, both abortive and prophylactic, for migraine headache have been shown to be inadequate for many patients due to lack of efficacy or intolerable side effects. This brings the focus to providing noninvasive therapies such as osteopathic manipulative treatment (OMT) to reduce migraine frequency and intensity. We aim to solidify the efficacy of OMT as an evidence-based treatment option by demonstrating underlying mechanisms and linking them to biomarkers of migraine pathophysiology. Here, we utilized adult female Sprague-Dawley (SD) rats to limit sex differences and reflect the gender susceptibilities in humans (female:male migraineurs ratio 2:1). Migraines were induced using a “double-hit” strategy starting with bilateral injections of Complete Freund Adjuvant (CFA, 10 µl/injection, 5 injections/side) to the trapezius muscle on

Day 1. CFA produced a mild inflammation, but the periorbital tactile threshold remained normal. On Day 8 after CFA-priming, umbellulone (50 mM in 50 µl), the volatile molecule constituting as a migraine trigger from the "headache tree," was introduced to awake rats via inhalation for 30 min in a small chamber with 2 L/min O_2 current. Soft tissue technique (STT), an osteopathic manipulative technique commonly used to treat migraines, was adapted to this rat model and compared against sham treatments. STT was applied at the dorsal aspect of the neck for 2 min under isoflurane anesthesia at D2 and 4 post-CFA. An additional treatment was applied at D8 immediately after umbellulone exposure. Periorbital tactile threshold was assessed prior to CFA administration and hourly for 5 h following umbellulone inhalation using calibrated von Frey filaments (cut off 8 g). The results showed that umbellulone successfully induced cephalic allodynia at 2-5 h post-dose selectively in CFA-primed rats, which was effectively mitigated by STT (P < 0.05 compared to the sham treatment). The evidence implies that OMT may function as an effective treatment for the headache and neck pains experienced during migraines. Further studies could elucidate the optimal amount of OMT necessary to replicate these results as well as OMT's effects on other biomarkers such as CGRP levels and restoration of normal physical activities using the voluntary running-wheel output measure.

Disclosures: K. Byrd: None. **B. Chung:** None. **K. Sharma:** None. **J. Xie:** None. **R. Fleming:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.08/H42

Topic: D.03. Somatosensation – Pain

Title: Next generation sequencing (NGS) in a case of severe migraine reveals a rare CACNA1A variant and highlights the complexity of NGS data implementation in clinical practice

Authors: *M. MAHALE[1], A. BASKYS[2];
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Abstract: Migraine, a disabling neurological disorder, affects~13% of the US population. Pathological mechanisms include mutations in *CACNA1A, SCN1A, PRRT2* and other genes. Triptans, carbonic anhydrase inhibitors, barbiturates and antiepileptics reduce migraine symptoms but their effectiveness is limited, likely due to a lack of specificity. NGS could identify pathogenic mutations and potentially increase treatment specificity, but is not routinely used clinically. To illustrate NGS use in clinical practice, we present a case of severe migraine with aura, nausea, vomiting, photophobia, central sleep apnea, confusion and episodic ataxia. The patient, a 26-year old male, was treated with zonisamide, rizatriptan, fluoxetine and

quetiapine without success. Symptoms begun 21 years ago and persisted throughout his life, negatively affecting its quality. There was no family history of neurological disorders. Whole Exome Sequencing (WES, www.Genos.co) identified 26,572 variants, 6 of which listed as 'pathogenic' in the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/). Variants were annotated using Ensemble VEP annotation engine (uswest.ensembl.org) and genes that contained variants found to be 'pathogenic', 'deleterious', or 'damaging' by predictor software were entered into VarElect (www.GeneCards.com) to calculate the degree of a gene association with terms 'nausea', 'vomiting', 'headache', 'migraine', 'photophobia'. The strongest association was found with *CACNA1A* gene coding for Ca^{2+} P/Q channel expressed in the cerebellum. The patient carried a rare (GnomAD allele frequency <0.0003) missense variant rs1196563175, nucleotide change c.6881G>C, in this gene that resulted in amino acid change p.R2294P. This change was deleterious/damaging by SIFT, PolyPhen, FATHM, M-CAP, Metal-R, MetaSVM, MutationTaster, PROVEAN, and had CADD=24.9 and DANN=0.964, suggesting pathogenicity. As *CACNA1A* variant carriers were reported to benefit from carbonic anhydrase inhibitor acetazolamide (AZ, Kinder et al, 2015), AZ treatment was initiated and found effective. To explore AZ interactions with *CACNA1A*, we compared AZ-regulated genes found in Comparative Toxicogenomics Database (ctdbase.org, CTD) with genes regulated by previously used ineffective drugs. *WNK1* gene, which regulates Na^+, K^+ and Cl^- transport as well as blood pressure and nerve conductance, was upregulated by AZ (Schreiner et al, 2009). No interactions of fluoxetine, razatriptan or zonisamide with *WNK1* have been reported. This case illustrates NGS utility in diagnosis and treatment of brain disorders and highlights complexities associated with clinical implementation of NGS data.

Disclosures: M. Mahale: None. **A. Baskys:** A. Employment/Salary (full or part-time):; MoodNote LLC, Educational Software Development Company.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.09/H43

Topic: D.03. Somatosensation – Pain

Support: NIH Grant CA196263

Title: Peripheral cannabinoid 1 receptor activation prevents hypersensitivity symptoms in murine models of chronic migraine and rebound headaches

Authors: *Y. MULPURI[1], T. YAMAMOTO[1], M. IZRAYLEV[1], C. KRAMME[1], M. SIMONIAN[1], H. H. SELTZMAN[2], I. SPIGELMAN[1];
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Abstract: Chronic migraine typically starts as an episodic disorder before progressing to the chronic phase. Triptans are highly effective in alleviating the symptoms of acute migraines, however, their repeated use leads to rebound headaches. Cannabinoids (CBs) suppress hypersensitivity associated with chronic pain states in animal models and human studies. Since CBs produce majority of their analgesic effects via peripheral cannabinoid receptor (CBR) activation, this eventually led to the development of several peripherally restricted cannabinoids (PRCBs) to circumvent potential psychotropic effects. Here we investigated if peripheral CBR activation with a PRCB, 4-{2-[-(1E)-1[(4-propylnaphthalen-1-yl)methylidene]-1H-inden-3-yl]ethyl}morpholine (PrNMI), can prevent the development of hypersensitivity in murine models of chronic migraine and rebound headaches. Female C57BL/6J mice were injected with nitroglycerin (NTG, 10 mg/kg, i.p, 7 doses, 2-day intervals) or sumatriptan (SUMA, 0.6 mg/kg, i.p, 7 doses, 2-day intervals) to induce baseline hypersensitivity to mechanical and cold stimuli, and following recovery from baseline hypersensitivity, latent sensitization to previously innocuous stimuli (0.1-0.3 mg/kg NTG). Administration of PrNMI (0.6 mg/kg, i.p) 1hr before (but not 1hr after) NTG injections decreased the development of acute and chronic hypersensitivity in the hindpaw and periorbital regions. PrNMI effects were mostly mediated by CB1R activation since co-administration of a CB1R antagonist completely (and a CB2R antagonist partially) reversed PrNMI effects. PrNMI (0.6 mg/kg) administered 90 min before SUMA injections completely prevented the development of basal hypersensitivity and the development of latent sensitization. Quantitative protein analysis revealed increased expression of protein kinase A (PKA) and phospho-PKA in dorsal root ganglia from NTG-treated mice, which was prevented by PrNMI pre-treatment. Trigeminal ganglia from SUMA-treated (but not PrNMI-pretreated) mice showed significant increases in the levels of nNOS and TRPA1 proteins compared to vehicle-treated mice. In patch-clamp recordings from dissociated trigeminal neurons of naïve mice, application of PrNMI (1 μM) reversibly decreased both low pH (6.0)- and allyl isothiocyanate (1 mM)-evoked currents via CB1R activation. In conclusion, targeting of peripheral CBRs with PRCBs may be a viable strategy for effective preventative treatment of these headache disorders.

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Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.10/H44

Topic: D.03. Somatosensation – Pain

Support: Fidelity Charitable Nancy Adams and Scott Schoen Fund
Kraig and Linda Kupiec Family Trust

Title: Short- and long-term neuromodulatory effects on migraine and trigeminal neuropathy pain

Authors: G. J. TOBIN[1], R. K. HARPER[1], D. SNODGRASS[2], F. YAN-GO[3], J. JEN[5], R. L. MERRILL[4], E. K. SAUERLAND[6], *R. M. HARPER[1];
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Abstract: Non-invasive neuromodulation of the sensory fields of cranial nerves V, VII, IX, and X, and cervical nerves of C2 and C3 results in significant declines in acute migraine and trigeminal neuropathy pain. Whether such pain declines can be maintained or follow a trend of reduced numbers or decreased attack intensity with repeated trials or prophylactic intervention use remain unclear. The neuromodulatory device consisted of bilateral silicon impressions of the auditory canals each containing a vibration device which reaches the cranial and cervical sensory fields. We examined multiple aspects of intervention outcomes 1) acute, first trial outcomes, 2) trends in pain or rate of attacks following repeated trials, 3) prevention of attacks after prophylactic use. All procedures were approved by the UCLA Institutional Review Board. Subjects were referred by UCLA or Mayo Clinic neurologists or pain specialists. In 56 subjects (N=56, female=37, male=18) with diagnosed migraine or trigeminal neuropathy pain, we evaluated changes in pain levels during 5 min sham vibration, followed by 15 min low amplitude vibration, 10 min high amplitude vibration, and a second 10 min sham vibration to cranial and cervical nerves. Pain was reduced from an average of 5.7 before stimulation to an average of 1.8 after stimulation ($p < 0.0001$) using self-perceived 0-10 pain level scales. Of these cases, 52 showed declines, and 4 showed no change (two of these 4 had no pain at onset); no increased pain levels were found after stimulation. Subsequent trials showed a gradual decline in pain intensity during attacks and a reduction in number of incidents. With prophylactic intervention, subjects used the devices at home 2-3 times weekly up to 2-3 times per day or during any signs of an aura. In one case, a subject with biweekly migraine attacks with blindness and 5 days of incapacity during each attack, migraines were (and continue to be) suppressed for 2 years with prophylactic use. Two hemicranial continua subjects with daily attacks have been migraine-free for more than 6 weeks. The collective data indicate both short- and long-term benefits from neuromodulation of cranial and cervical nerves, and potential sustained migraine suppression with prophylactic use.

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Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.11/H45

Topic: D.03. Somatosensation – Pain

Support: Medical Research Council
The Migraine Trust

Title: Environmental and genetic circadian disruption increases migraine-associated phenotypes in mice

Authors: *L. C. STROTHER[1], L. J. PTACEK[2], P. J. GOADSBY[1], P. R. HOLLAND[1]; [1]King's Col. London, London, United Kingdom; [2]Dept Neurol, UCSF, HHMI, San Francisco, CA

Abstract: OBJECTIVES Migraine is strongly linked to variation and disruption in circadian rhythms: migraine attack onset is highest in the morning, jet lag and shift work trigger migraine, and a genetic mutation in the molecular clock regulatory kinase, casein kinase 1 δ (CK1δ), is comorbid for migraine with aura and Familial Advance Sleep Phase (FASP). To investigate this link preclinically, we sought to explore the effect of circadian rhythms and their disruption on periorbital (PO) allodynia in mice. We also sought to characterize migraine-like phenotypes in transgenic mice harboring not only the CK1δ mutation, but also a second mutation for FASP affecting the clock specific protein Per2 in order to identify if clock disruption alone is sufficient to induce migraine-like phenotypes. **METHODS** In C57bl/6J mice (*n*=24), PO mechanical allodynia was measured using the von Frey assay at zeitgeber time (ZT) 0, 6, 12, 18; chronic jet lag was modelled by changing light cycles and mechanical sensitivity assessed at ZT0 and 12. Migraine-like phenotypes including cortical spreading depression (CSD) and nitroglycerin-induced PO allodynia were assessed in CK1δ-T44A (*n*=24), Per2-S662G (*n*=25) transgenic mice and matched WT littermates (*n*=47). CSD was induced in anaesthetized mice using constant stimulus potassium chloride (1M) and CSD events recorded for 1 hr. PO allodynia was measured at baseline and again 2 hrs post nitroglycerin treatment (5mg/kg i.p.) **RESULTS** PO mechanical sensitivity exhibits circadian variation with the lowest threshold observed at ZT0 (0.48 ± 0.06) and highest at ZT12 (1.01 ± 0.07). Additionally, thresholds were significantly lower at both time points after changing light cycles compared to control (ZT0: $t_{44} = 2.74$, $p < 0.05$; ZT12: $t_{44} = 5.87$, $p < 0.0001$). Both transgenic lines showed significant increase in CSD events compared to WT (WT=7, CK1δ-T44A=10; U=12, $p<0.0001$) (WT=6, Per2-S662G=7; U=30, $p<0.05$). Transgenic mice also showed an increase in PO mechanical sensitivity following nitroglycerin treatment (WT vs CK1δ-T44A: $F_{1,22}=41.61$; $p<0.0001$; WT vs Per2-S662G: $F_{1,22}=32.13$; $p<0.0001$) **CONCLUSION** C57bl/6J mice exhibit circadian variation in PO mechanical sensitivity suggestive of a circadian rhythmicity in sensory processing that could underlie the circadian nature of migraine attack onset. Additionally, exposure to changing light cycles impacts on migraine relevant pain processing. Furthermore, disruption to the clock protein Per2 is, at least in part, sufficient to induce increased susceptibility to migraine-associated phenotypes in preclinical models, which for the first time, implicates a purely circadian gene in migraine pathophysiology.

Disclosures: L.C. Strother: None. **L.J. Ptacek:** None. **P.J. Goadsby:** None. **P.R. Holland:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.12/H46

Topic: D.03. Somatosensation – Pain

Title: Oxytocin modulates the nociceptive transmission at trigeminocervical complex evoked by electrical meningeal stimulation

Authors: *J. E. GARCIA-BOLL, G. MARTÍNEZ-LORENZANA, A. GONZÁLEZ-HERNÁNDEZ, M. CONDÉS-LARA;
Inst. of Neurobio., Queretaro, Mexico

Abstract: ,

Disclosures: J.E. Garcia-Boll: None. **G. Martínez-Lorenzana:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.13/I1

Topic: D.03. Somatosensation – Pain

Title: Does heat shock protein 90 have a role in regulating the blood brain barrier during cortical spreading depression?

Authors: *S. M. PALOMINO[1], J. M. STREICHER[2], T. M. LARGENT-MILNES[1];
[2]Pharmacol., [1]Univ. of Arizona, Tucson, AZ

Abstract: The classical function of the blood-brain barrier (BBB) is the regulation of the transcellular and paracellular transport between the CNS and the vasculature. Recent studies have shown that the BBB can become dysregulated during a cortical spreading depression (CSD) event. CSD events could change analgesic efficacy or lead to CNS toxicity of anti-migraine therapeutics, including first-line therapies like triptan compounds. The clarification of biochemical mechanisms regulating CSD-induced changes in BBB function is crucial to understand the efficacy of antimigraine drugs. In order to investigate the BBB changes caused by CSD events we established an *in vivo* model of episodic headache utilizing a dural cannulation

method and *in vitro* models of the neurovasvular unit. Cortical KCl injection decreased the expression level of NHE1 protein, a proton exchanger, which may alter the uptake kinetics of sumatriptan, NHE1 has a primary role in regulating intracellular pH and can be implicated in contributing to migraine. New studies performed in the lab have suggested that dysregulation of NHE1 could be due to decreased phosphorylation at the C- terminal domain of the NHE1 protein complex. These events may be linked to another molecular protein involved in chaperone and kinase functions, Heat shock protein 90 (HSP90). We investigated further using western blotting methods and observed a decreased expression of HSP90 in Trigeminal Ganglion (TG) samples taken from KCl treated brains. NHE1 phosphorylation and function have been linked to the AKT pathway. We utilized immortalized (bEND3) cells with Astrocyte conditioned media as an *in vitro* model of the neurovascular unit. In our model, 17AAG (Hsp90 inhibitor) treatment considerably reduced total and phosphorylated levels of the AKT protein kinase . We further investigated HSP90 inhibition effects on intracellular pH to compare its effects to KCl treated cells. Also, we will investigate the effects of 17AAG on paracellular transport of sucrose as well as intracellular transport of sumatriptan across our *in vitro* model of the BBB. Furthermore, we will investigate the effects of 17AAG treatment *in vivo* to observe if HSP90 inhibition may lead to increased periorbital allodynia using Von Frey method. This study thus promises to advance our understanding of BBB integrity during CSD events and determine how CNS uptake of antimigraine therapeutics is regulated during migraine attacks as well as possibly illuminate a molecular mechanism which may reveal the NHE1 protein to be majorly involved in a mechanism of CSD dysregulation of the BBB.

Disclosures: S.M. Palomino: None. **J.M. Streicher:** None. **T.M. Largent-Milnes:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.14/I2

Topic: D.03. Somatosensation – Pain

Support: NIH DE024629

Title: Dietary supplementation with grape seed extract prevents development of trigeminal sensitization and inhibits pain signaling in a preclinical chronic TMD model

Authors: L. CORNELISON[1], S. E. WOODMAN[1], J. COX[1], C. LIKENS[1], H. CHILDS[2], S. ANTONOPOULOS[1], ***P. L. DURHAM**[1];
[1]Missouri State Univ., Springfield, MO; [2]MISSOURI STATE UNIVERSITY, SPRINGFIELD, MO

Abstract: Objective: To investigate the effects of dietary supplementation of grape seed extract (GSE) on the development of a sensitized trigeminal system and pain signaling in a chronic temporomandibular joint disorder (TMD) model. Additionally, changes in the expression of proteins associated with the development of trigeminal sensitization were investigated. Background: Risk factors such as neck muscle tension, female gender, and prolonged jaw opening, which occurs during routine dental or orthodontic procedures, increase the likelihood of developing chronic TMD. Peripheral and central sensitization, which is promoted by changes in expression of proteins and cytokines that facilitate enhanced neuron-glia communication, are implicated in the pathology of chronic TMD. In a prior study, inclusion of GSE elevated basal expression of proteins that decrease neuronal excitability and suppressed expression of several proteins implicated in trigeminal sensitization. Methods: Mechanical nocifensive thresholds were determined in male and female Sprague Dawley rats using von Frey filaments. To promote trigeminal sensitization, animals were injected with complete Freund's adjuvant in the trapezius. After 8 days, animals were subjected to proloned jaw opening and head withdrawal responses were determined for 28 days. Some animals received GSE via their drinking water 2 weeks prior to trapezius injections. Animals were sacrificed at various timepoints and tissues collected to investigate molecular changes in neuronal and glial cells in the spinal cord and trigeminal ganglion using immunohistochemistry, qPCR, and cytokine/chemokine arrays. Results: Muscle inflammation and jaw opening in males increased the average number of nocifensive responses for 21 days, however in females, sensitivity persisted for 28 days. GSE significantly suppressed development of a sensitized state and inhibited pain signaling in response to prolonged jaw opening. Surprisingly, increased expression of the proteins CGRP, PKA, NF-kB, GFAP, and Iba1, which are implicated in central sensitization, was not observed in the dorsal horn. However, mRNA expression of Cx26 in trigeminal ganglion and Cx26, Cx43, Cx36, Cx40, pannexins 1 and 2 in the spinal trigeminal nucleus was increased, and the level of multiple cytokines was elevated. Conclusions: Our findings provide evidence that dietary inclusion of GSE can prevent trigeminal pain signaling and thus could be useful in the management of TMD. Furthermore, the prolonged nociception observed in our TMD model is likely mediated by changes in expression of connexins, pannexins, and cytokine levels to promote trigeminal sensitization.

Disclosures: P.L. Durham: None. **L. Cornelison:** None. **S.E. Woodman:** None. **J. Cox:** None. **C. Likens:** None. **H. Childs:** None. **S. Antonopoulos:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.15/I3

Topic: D.03. Somatosensation – Pain

Support: International Dehydrated Foods TA 19 18

Title: Trigeminal sensitization caused by early life stress is inhibited by dietary supplementation with enriched chicken bone broth in a model of episodic migraine

Authors: O. PETERSON, ***L. CORNELISON**, P. L. DURHAM;
Missouri State Univ., Springfield, MO

Abstract: Objective:
The focus of this study was to determine the effects of early life stress and dietary inclusion of enriched chicken bone broth (ECBB) on trigeminal nociceptor sensitization and activation in a model of episodic migraine.
Background:
Early life stress is a risk factor for development of migraine, a common orofacial pain disease characterized by activation of trigeminal nociceptors and intense headache, which can be initiated by pungent odors in sensitized individuals. Recently, dietary supplementation with enriched chicken bone broth was shown to attenuate trigeminal nociception in a model of temporomandibular joint disorder.
Methods:
Adult Sprague-Dawley male sender rats subjected to primary traumatic stress via repeated forced swimming were placed next to breeding or pregnant female rats that served as receiver rats (secondary traumatic stress) and in proximity to the offspring after weaning. When offspring reached adulthood, unstressed and stressed young adult male and female animals (~day 56) were tested for basal nocifensive response to mechanical stimulation over the masseter and temporalis using von Frey filaments. Mechanical sensitivity was also tested in response to brief exposure to a pungent oil extract of the California bay leaf that is reported to be a migraine trigger, and in response to dietary supplementation of an enriched chicken bone broth (1% dissolved in drinking water).
Results:
Early life stress promoted a sensitized state of trigeminal nociceptors that were activated by the pungent CBL extract in both sexes when compared to unstressed control animals. Female animals exhibited a higher basal nociceptive level and prolonged sensitization compared to males. Dietary supplementation of enriched chicken bone broth at the time of weaning inhibited both basal and odor-triggered trigeminal nociception.
Conclusions:
Early life stress promoted development of a persistent sensitized trigeminal system that is more severe in females, which is characteristic of migraine pathology. Inclusion of enriched chicken bone broth as a dietary supplement suppressed trigeminal sensitization, and thus may provide a nutraceutical method for reducing migraine risk mediated by early life stress.

Disclosures: O. Peterson: None. **L. Cornelison:** None. **P.L. Durham:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.16/I4

Topic: D.03. Somatosensation – Pain

Support: Electrocore

Title: Noninvasive vagus nerve stimulation inhibits trigeminal nociception by enhancing descending pain modulation: similar effect as observed with morphine in chronic migraine model

Authors: *S. E. WOODMAN, L. CORNELISON, P. L. DURHAM;
Missouri State Univ., Springfield, MO

Abstract: Objective: To delineate the cellular mechanism involved in non-invasive transdermal vagus nerve stimulation (nVNS) inhibition of trigeminal nociception in an episodic migraine model and compare the analgesic effectiveness of nVNS to morphine in a chronic migraine model. **Background:** Migraine is a debilitating neurovascular disorder characterized by recurring moderate to severe headache attacks. Opioid over-prescription, overuse, and addiction is a crisis, creating an impetus to explore alternative, non-addictive therapies. Non-invasive vagus nerve stimulation (nVNS) has proven efficacious in human migraine patients and could be a promising alternative or adjunctive therapeutic. **Methods:** For the episodic migraine model, neck muscle inflammation was induced via injection of complete Freund's adjuvant (CFA) in the trapezius in male Sprague Dawley rats. Eight days post CFA, trigeminal neurons were activated by exposure to a pungent California bay leaf extract (CBL). To ameliorate nociception, animals received nVNS stimulation. Nocifensive head withdrawal response was investigated using von Frey filaments. To investigate the mechanism of nVNS, some animals were injected intracisternally with the $GABA_A$ antagonist Bicuculline, the $5\text{-}HT_3$ antagonist Ondansetron, or the $5\text{-}HT_7$ antagonist SB-269970 just prior to nVNS. For the chronic migraine model, animals received CFA injections and one night of REM sleep deprivation 8 days prior to CBL exposure. On day 3 following CBL exposure, animals were treated with either nVNS or morphine for seven days. **Results:** Neck muscle-sensitized animals displayed increased nociception, which was attenuated by nVNS 2 hours post-trigger and remained repressed. Injection of antagonists of $GABA_A$ receptors or $5\text{-}HT_3$ and $5\text{-}HT_7$ receptors blocked the analgesic effect of nVNS. Exposure of animals sensitized by neck inflammation and REM sleep deprivation to CBL resulted in a heightened state of nociception that persisted for >21 days. Morphine and nVNS administration similarly repressed trigeminal nociceptor sensitization in a transient manner in the chronic migraine model. **Conclusions:** Our findings provide evidence that nVNS inhibits trigeminal activation via a mechanism involving enhanced descending pain modulation, which differs from the mechanism reported for triptans, and that nVNS was similarly effective as morphine in

inhibiting trigeminal nociception in a model of chronic migraine. Thus, we propose that nVNS may offer a novel therapeutic option for triptan non-responders, and furthermore nVNS may be beneficial as an adjunct or replacement opioid therapy.

Disclosures: S.E. Woodman: None. **L. Cornelison:** None. **P.L. Durham:** None.

Poster

053. Somatosensation: Headache and Migraine

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 053.17/I5

Topic: D.03. Somatosensation – Pain

Title: Long lasting pain inhibition by C tactile afferents

Authors: *S. SHAIKH[1], H. OLAUSSON[1], M. SAVALLAMPI[1], F. P. MCGLONE[2], S. S. NAGI[1];
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Abstract: We had previously shown that slow, pleasant brushing at a velocity (3 cm/s) optimal for activation of unmyelinated C-tactile fibres (CTs), reduces heat pain in humans (Liljencrantz et al. 2017). However, we also know that pleasant brushing can evoke allodynia in other models of experimental pain, e.g. hypertonic saline infusion (Nagi et al. 2011). Here, we investigate the effect of CT-targeted brushing on acute pressure pain & the spatio-temporal characteristics of pain inhibition. Using a rotatory tactile stimulator (RTS), continuous brushing was delivered for at least 5 min (≤ 8 min) with a force of 0.4 N & a CT-targeted velocity of 3 cm/s on the hairy skin of the right forearm. Using a digital algometer, pressure pain thresholds (PPTs) were measured on the right & left palm muscles, ipsilateral & contralateral to brushing respectively. PPTs were recorded before & during brushing, & every 5 mins for one hour after the brushing had stopped. In the non-brushing condition, application of pressure to measure PPTs every 5 mins produced a 'wind-up' due to temporal summation which decreased baseline PPT values over time. In the brushing condition, we found a significant increase in PPTs within 80-120 seconds of the cessation of brushing. The 'wind up' was reversed when the PPT trials were preceded by brushing with the pain inhibition lasting for up to an hour. The pain relief was limited to the ipsilateral side & did not extend to contralateral or remote sites. In conclusion, CT-targeted brushing reduces pressure pain in a somatotopically constrained manner. The pain-alleviating effect of CT-targeted touch started soon after the brushing had stopped & lasted for up to an hour, showing a touch medaited long-lasting inhibition of pain. The effect observed here is consistent with recent observations in rodent models of neuropathic pain of a low-threshold C-fibre (C-LTMR) specific inhibitory pathway for long-lasting analgesia - activated by the release

of TAFA4, a chemokine-like protein found only on these afferents (Kambrun et al. 2018; Delfini et al. 2013).

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Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.01/I6

Topic: D.03. Somatosensation – Pain

Support: NNSFC grant 84870872, 31270882

Title: Effect of protein disulfide isomerase in DRG on mice pain behaviors

Authors: *Y. ZHANG[1], H. ZHANG[2], X. DU[3];
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Abstract: Objective: PDI (PDIA1) is the prototype member of protein disulfide isomerases (PDIs) family.
It has been implicated to play a role in pain behavior. The present study is aimed to determine the roles of PDI in regulating pain behavior in mice dorsal root ganglion (DRG).
Methods: First, we used immunofluorescence method to explore the expression of PDI in DRG tissue. Second, western blot test was employed to quantify the protein change. Finally, DRG-specific PDI knock out mice were subjected to Hargreaves and von Frey test.
Results: Firstly, immunofluorescent results showed that PDI highly expressed in DRG neuron and partially colocalized with marker IB4 (isolectin B4), CGRP (calcitonin gene related peptide) and NF200 (neurofilament-200). Secondly, PDI protein increased markedly in DRG tissue under chronic pain murine models of CCI (chronic constriction sciatic nerve injury) and CFA (Complete Freund's adjuvant). Lastly, DRG-specific PDI knock out mice exhibited reduced pain thresholds in CCI neuropathic pain model and CFA inflammatory model.
Conclusions: In chronic pain conditions, PDI protein increased significantly to maintain or contribute to pain progress. It may be a new target to treat pain.
Key Words: pain PDI DRG CCI CFA

Disclosures: Y. Zhang: None. **H. Zhang:** None. **X. Du:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.02/I7

Topic: D.03. Somatosensation – Pain

Support: KAKENHI 19J14595
KAKENHI 17h03933

Title: The activation of alpha 2 adrenergic receptors inhibits pain behavior related to TRPV1 at the peripheral nerve level

Authors: *Y. MATSUSHITA, M. MANABE, N. KITAMURA, I. SHIBUYA;
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Abstract: Animals have mechanisms of not only pain detection but pain relief. In the central nervous system, the descending antinociceptive system (DAS) is a typical analgesic mechanism and noradrenaline (NA) is one of the most important neurotransmitters in the DAS. Analgesia by adrenergic systems in the central nervous system, but not in the peripheral nervous system, has been well investigated. Recently, we examined the functional association between NA and transient receptor potential vanilloid 1 (TRPV1), one of the pain sensing molecules, and reported that NA inhibited capsaicin-evoked TRPV1 activities though α_2 adrenergic receptors in rat dorsal root ganglion neurons. In this study, we investigated effects of clonidine, an α_2 adrenergic receptor agonist, on pain behavior evoked by TRPV1 activation by capsaicin, noxious heat and formalin in rats. Capsaicin injection into the skin of the plantar surface of the hind paw induced pain behavior and it was reduced by pre-injection of clonidine into the same site of capsaicin injection. This inhibitory effect was not observed when clonidine was injected into hind paws on the contralateral side. The inhibitory effect of clonidine on capsaicin-induced pain behavior was prevented by yohimbine, an α_2 adrenergic receptor antagonist. Since TRPV1 is also activated by the noxious heat, we measured latency to withdrawal responses to heat stimulations by Hargreaves method. Pre-injection of clonidine into the ipsilateral hind paws where heat stimulations were applied increased withdrawal latency. This action of clonidine was not observed when clonidine was injected on the contralateral side. Clonidine injection into the ipsilateral hind paws also reduced pain behavior observed in the formalin test in both Phase I (early) and II (late) significantly. No inhibitory effect of clonidine on formalin-induced pain behavior was observed when clonidine was injected on the contralateral side. These results suggest that the activation of α_2 adrenergic receptors inhibits TRPV1 activity at the peripheral nerve level of the primary sensory neuron *in vivo*, resulting in analgesia.

Disclosures: Y. Matsushita: None. **M. Manabe:** None. **N. Kitamura:** None. **I. Shibuya:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

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Program #/Poster #: 054.03/I8

Topic: D.03. Somatosensation – Pain

Support: ANR-17-CE16-0018-02

Title: Peripheral ASIC3 activation by lysophosphatidylcholine induced chronic joint pain and associated anxiety in mice

Authors: *F. JACQUOT[1], J. BARBIER[1], A. BAYLE[1], L. DELAY[1], E. LINGUEGLIA[2], D. ARDID[1], E. DEVAL[2], F. MARCHAND[3];
[1]Neuro-Dol, Clermont-Ferrand, France; [2]CNRS/UNSA UMR6097, Valbonne, France; [3]INSERM, Clermont Ferrand, France

Abstract: We have shown previously that human painful inflammatory exudates, displaying nonacidic pH, induced a slow constitutive activation of human ASIC3 channels. This effect was mainly driven by lipids, and we identified lysophosphatidylcholine (LPC) and arachidonic acid (AA) as endogenous activators of ASIC3 in the absence of any extracellular acidification. The combination of LPC and AA also evoked strong depolarizing current in rat DRG neurons at physiological pH 7.4. Our aim was to further assess the pronociceptive *in vivo* effect of intraarticular LPC in mice and to investigate associated comorbidities, especially anxiety. We evaluated if ASIC3 channel was involved in these effects using ASIC3 knock out mice. Finally, the role of ASIC1a channel of the basolateral amygdala (BLA) in LPC-induced pain behaviors and anxiety was studied. We demonstrated that two intraarticular LPC injections (10nM) 5 days apart induced significant mechanical and thermal hypersensitivities up to 28 days compared to control mice. This was not associated with peripheral inflammation or bone remodeling using MMP680 and Cathepsin K *in vivo* fluorescent imaging, respectively, as well as neuronal damage. LPC-injected mice also develop anxiety demonstrating by several anxiety tests (open field, elevated plus maze, hole board, marble burying). Furthermore, pain behaviors and associated anxiety induced by local LPC were significantly reduced in male and female ASIC3 knock-out mice compared to their WT counterpart. Finally, blockade of ASIC1a following a microinjection of PcTx1, a specific blocker of ASIC1a, within the BLA, significantly reduced anxiety but not mechanical hypersensitivity following intraarticular LPC. This effect was associated to a significant decrease of Fos expression in this structure. Here, we demonstrated that intraarticular LPC induced chronic pain behaviors and anxiety related behaviors in an ASIC3 dependent manner in mice. These results suggest that lipids especially LPC through ASIC3 activation seems to play a crucial role in the development of chronic joint pain from different etiologies and could be a valuable therapeutic target.

Disclosures: **F. Jacquot:** None. **J. Barbier:** None. **A. Bayle:** None. **L. Delay:** None. **E. Lingueglia:** None. **D. Ardid:** None. **E. Deval:** None. **F. Marchand:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.04/I9

Topic: D.03. Somatosensation – Pain

Title: Intradermal capsaicin induced paw flinch count is a highly reproducible model of acute pain in rat

Authors: *H. RASHID, R. SAMADFAM;
Charles River Labs, Senneville, QC, Canada

Abstract: Introduction: The hot chili pepper ingredient capsaicin, which is also an agonist for the transient receptor potential vanilloid type 1 (TRPV1), can induce spontaneous non-evoked pain reactions as well as neurogenic inflammation and hyperalgesia when injected intra-dermally in both human and rodents. The TRPV1 channel is also known as a common transducer of pain for multiple exogenous and endogenous algogens and as such has been a target for development of analgesic agents for long time. In spite of its high translational value, there are limited reports for the peripheral capsaicin-induced pain model in rodents, especially in terms of its reproducibility. In the present study, we optimized the model for counts of paw flinches following intra-dermal capsaicin injection and showed reproducibility of the model over a number of experiments. **Methods**: Capsaicin solution, dissolved in 20% ethanol, 7% Tween80 and 73% saline, was injected intra-dermally into the left hind paw glabrous skin of the rat and capsaicin-evoked spontaneous nociceptive behaviors in rats were continuously recorded for 0-10 minutes using a commercial camcorder. Scoring of the total number of flinches of the injected paw was counted (off-line) using the recorded video file. Tramadol was used a positive control and administered by oral gavage at 30 minutes before capsaicin injection. **Results**: Injection of 0.01% capsaicin induced flinching behaviors in the rat while time spent on biting and licking of the injected paw was minimal. Dose-dependent flinching counts were also observed when 0.01%, 0.05% and 0.1% capsaicin solutions were injected. In subsequent experiments, 0.01% and 0.05% capsaicin solutions were selected for use. The time-course of 0.01% capsaicin-induced flinch counts shows that peak of the pain behaviors occur mainly within 1-3min post injection which then gradually dissipate by 10 minutes post injection. Data from 10 subsequent experiments using 0.01% capsaicin showed that flinch counts could be used as a reproducible endpoint for measuring peripheral acute pain in rat. The standard analgesic drug tramadol (60 mg/kg, PO) significantly blocked the capsaicin-evoked spontaneous flinching reactions in the rats. **Conclusion**: Overall, the present results showed that counting of flinches following intra-

dermal capsaicin injection could be reproducibly used as a peripheral acute pain model in rat. Considering the multi-modal nature of the TRPV1 transducing receptor and, the simplicity and reproducibility of the model, it could be used for rapid screening of analgesia targeted drugs.

Disclosures: H. Rashid: None. **R. Samadfam:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.05/I10

Topic: D.03. Somatosensation – Pain

Support: NRF-2017R1C1B5076722

Title: Cinnamaldehyde produce anti- nociception via AQP5 and pERK1/2 signaling in rats with xerostomia

Authors: *M. LEE[1], J. CHOI[2];
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Abstract: Xerostomia is a relatively common oral symptom, resulting in various problems such as pain, mastication discomfort and tissue injury. When the activity of AQPs decreases, salivary secretion decreases and dry mouth syndrome occurs. The 4-DAMP used to construct the model of xerostomia is a drug that acts antagonist to muscarinic M3 receptor distributed in the submandibular gland to suppress parasympathetic nerve and decrease secretion of saliva. pERK is known to play an important role in inflammation and pain development and maintenance. Xerostomia can cause not only inflammation of tissues but also orofacial pain or enhanced pain, but the relevance of pERK pathway to xerostomia induced orofacial pain has not yet been established. Cinnamaldehyde is a potent antioxidant and antiinflammatory drug. In this study, we constructed a xerostomia model based on the salivary gland injury by intraperitoneal injection of 4-DAMP, and observed changes in the submandibular tissue and the expression level of AQP5 and pERK in salivary glands or medulla oblongata. In addition, oral administration of cinnamaldehyde resulted in enhanced orofacial pain by xerostomia and gland tissue damage recovery level of submandibular tissue or medulla oblongata. To establish the xerosomia model, intraperitoneal doses of 1 ml of 4-DAMP (1 mg/kg) and 20% urethane (0.5 ml/kg) are administered to experimental animals. After placing a cotton ball on the mouth of the submandibular gland for 30 minutes, weight of the cotton ball was regarded as the amount of absorbed. Cinnamaldehyde (5, 12.5, 25 and 50 mg/kg) was first dissolved in 20% DMSO and diluted in distilled water to 1 ml. In the xerostomia model, 5% formalin (30, 50 μL) was subcutaneously injected into the TMJ and vibrissa pad of the rat model to observe the change in threshold of the pain model. In the xerostomia by 4-DAMP, the between glandular tissues

improved in the submandibular glands were wider than those of the control group. In the quantitative analysis of protein, expression of AQP5 was decreased in the 4-DAMP group compared with the naïve group, and the expression level of cinnamaldehyde was increased in the group. pERK level was increased in the enhanced pain group with xerostomia at the medulla oblongata and decreased significantly by cinnamaldehyde. Based on these results, the administration of 4-DAMP resulted in histological and biochemical changes for xerostomia, which could be a factor to increase orofacial pain. Cinnamaldehyde also modulates xerostomia, and may be used as a potential remedy for improved orofacial and TMJ pain relief.

Disclosures: M. Lee: None. **J. choi:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: K01 DA042902
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Title: Comparison of fentanyl, buprenorphine, and DAMGO analgesic efficacy and potassium flux in mice lacking K_{ATP} channel activity

Authors: *G. T. SAKAMAKI, K. JOHNSON, M. MENSINGER, A. H. KLEIN;
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Abstract: There are several classes of opioids targeting the mu opioid receptor, each with various functional selectivity of downstream pathways including K_{ATP} channels. As part of an on-going investigation into differences between synthetic, semi-synthetic, and non-synthetic opioids and their downstream effects on potassium channel signaling, we hypothesize there are differences in K_{ATP} channel activation between buprenorphine, fentanyl, and [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO). Male and female mice lacking the SUR1-K_{ATP} channel subunit (SUR1 KO) were behaviorally tested against wild-type (SUR1 WT) and heterogenous (SUR1 Het) using modified Hargreaves and von Frey methods to measure thermal and mechanical nociception, respectively. Latency and force measurements were taken before and after drug injection (DAMGO, 10 mg/kg; Fentanyl, 0.25 mg/kg; Buprenorphine, 5.83 mg/kg) at 3 min., 15 min., 30 min., 45 min., 60 min., and 120 min., post subcutaneous injection. Potassium channel flux was measured using a fluorescence intensity plate reader in a neuroblastoma immortal cell line (SH-SY5Y), and cultured primary dorsal root ganglia from SUR1 KO, WT, and Het mice. Preliminary data from behavior testing in mice show differences in the magnitude

and length of the thermal and mechanical paw withdrawal latencies/forces in SUR1 WT vs. SUR1 KO mice after opioid drug administration. Behavior testing for DAMGO had similar effects on SUR1 KO and WT mice but increased on SUR1 Het mice, while fentanyl had a decreased effect on mechanical nociception of SUR1 KO vs. SUR1 WT mice. Fentanyl caused an immediate analgesic efficacy peak at 3 min. post-injection decreasing over time on thermal nociception of SUR1 WT and KO mice but SUR1 Het mice had an increasing analgesic effect with the efficacy peaking 60 mins. post-injection. Potassium flux increased in a dose-dependent manner after fentanyl and buprenorphine administration in cultured SH-SY5Y cells but increasing DAMGO concentrations did not increase potassium flux. We conclude that there are differences in K_{ATP} channel dependent activity between fentanyl, buprenorphine, and DAMGO from our behavioral assays and fluorescence intensity data. Future experiments will possibly investigate the K_{ATP} channel contribution of analgesia in the central nervous system, utilizing tissue cultures from the spinal cord or higher order brain regions.

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Poster

054. Pain: Animal Models of Behavior

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Topic: D.03. Somatosensation – Pain

Support: Grant Linking University wide expertise from the Indiana Clinical Translational Sciences Institute
CA200417

Title: Vincristine treatment during adolescence produces peripheral neuropathy that persists into adulthood in a rat model

Authors: *A. LI[1], G. RAJIC[1], L. M. CAREY, IV[1], J. D. CRYSTAL[1], Y. Y. LAI[1], T. J. SAJDYK[2], J. L. RENBARGER[2], A. G. HOHMANN[1];
[1]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Pediatrics Hematology/Oncology, Indiana University, Sch. of Med., Indianapolis, IN

Abstract: Childhood acute lymphoblastic leukemia (ALL) is a significant clinical problem that can be effectively treated with vincristine, a vinca alkaloid-based chemotherapeutic agent. However, due to the increased survival rate, nearly all children receiving vincristine treatment develop dose-limiting sensory, motor and/or autonomic peripheral neuropathy[1][1,2][1] We previously reported that pediatric ALL populations are highly vulnerable to developing vincristine-induced peripheral neuropathy (VIPN) [1][1,3][1]. The possible long term impact of

adolescent vincristine treatment across the lifespan remains incompletely understood. We, consequently, developed an adolescent rodent model of VIPN which can be utilized to study both the mechanisms underlying VIPN and possible long term consequences of vincristine treatment in the developing rat. Adolescent rats of both sexes received once daily intraperitoneal injection of vincristine for 15 days during the critical period of adolescence (i.e. postnatal day P35 to P49). Separate groups received saline injections in parallel or were left untreated. Mechanical paw withdrawal thresholds, duration of responding to cold, rotarod descent latency, and grip strength (all paws and forepaws) were assessed before vincristine treatment and once every four days until adulthood P71. Body weight was also monitored throughout the experiment. Vincristine-treated animals developed hypersensitivity to mechanical and cold stimulation of the plantar hind paw surface. Hypersensitivity to mechanical and cold stimulation developed shortly following the onset of the vincristine dosing regimen and outlasted the period of vincristine treatment for approximately 10 days. However, both mechanical and cold allodynia resolved by P63. No differences in rotarod performance were observed between saline- and vincristine- treated animals. Vincristine-treated animals exhibited delayed increases in grip strength (all paws) compared to the saline-treated group from P54 to P70. No differences were observed in forepaw grip strength between vincristine- and saline-treated groups. Interestingly, voluntary running wheel exercise attenuated development of vincristine-induced hypersensitivities to mechanical and cold stimulation. Our studies support a role for nonpharmacological strategies such as voluntary exercise in the management of unwanted side effects of chemotherapeutic treatment. Supported by a Grant Linking University Wide Expertise (to AGH and JR) and CA200417 (to AGH)

Disclosures: A. Li: None. **G. Rajic:** None. **L.M. Carey:** None. **J.D. Crystal:** None. **Y.Y. Lai:** None. **T.J. Sajdyk:** None. **J.L. Renbarger:** None. **A.G. Hohmann:** None.

Poster

054. Pain: Animal Models of Behavior

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS080889

Title: Early life vincristine exposure does not prime developing pain pathways

Authors: *L. M. STYCZYNSKI, K. A. SCHAPPACHER, E. K. SERAFIN, M. L. BACCEI; Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Our recent work has demonstrated that the early life administration of vincristine (VNC), commonly used to treat pediatric leukemias, evokes peripheral neuropathy and

mechanical pain hypersensitivity in rats that persists into adolescence. However, whether neonatal VNC treatment alters baseline pain sensitivity throughout adulthood remains unknown. It is also unclear if pediatric VNC exposure can 'prime' developing nociceptive pathways, and thereby exacerbate chronic pain, following subsequent trauma later in life. Therefore, the present study investigated the degree to which neonatal VNC administration influences mechanical and thermal pain hypersensitivity in the absence or presence of tissue injury sustained during adulthood. Rats received five total doses of 60 μg/kg VNC (or vehicle) on postnatal days (P) 11, 13, 17, 19 and 21, which is known to evoke mechanical pain and skin denervation by P26 (Schappacher et al., 2017). Immunohistochemical analysis of the skin suggested that a resolution of the peripheral neuropathy occurs by 13-15 weeks of age, as there were no differences in the density of intraepidermal nerve fibers or activated Langerhans cells between the VNC and vehicle-treated groups at this time point. Baseline mechanical pain sensitivity, as measured with von Frey hairs, was also similar between the experimental groups during adulthood, confirming recovery to neonatal VNC treatment. To explore the potential long-term effects of early life VNC on the behavioral response to subsequent insults, a surgical incision of the hindpaw was administered to adult rats exposed to either VNC or vehicle during the neonatal period. We observed no significant overall effect of early life VNC on the magnitude of post-operative pain following adult incision. In addition, in order to model the clinical scenario where cancer relapse necessitates a second round of chemotherapy, separate groups of rats that had been treated with VNC (or vehicle) as neonates were subsequently administered VNC during adulthood (five injections at 100 μg/kg). There was no significant effect of prior VNC exposure on the level of mechanical pain hypersensitivity produced by adult VNC treatment. Collectively, these findings suggest that neonatal VNC does not increase the susceptibility to develop chronic pain as adults.
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Poster

054. Pain: Animal Models of Behavior

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Topic: D.03. Somatosensation – Pain

Support: NIH NIGMS grant P20GM103643

Title: Paclitaxel-induced peripheral neuropathy in rodents: Standardization of protocol with respect to sex, species, and paclitaxel formulation

Authors: D. GIUVELIS, I. D. MENG, ***K. L. TUCKER**;
Univ. of New England, Biddeford, ME

Abstract: Paclitaxel, commonly known as Taxol, is a chemotherapy medication used for a wide range of cancers, including pancreatic, cervical, breast, ovarian and lung cancer. Paclitaxel-induced peripheral neuropathy (PIPN) is a common side effect of chemotherapy, which can begin within days of a first dose and has been shown to affect as many as 60-70 percent of chemotherapy patients. PIPN can cause bilateral symptoms such as pain, paresthesia, or numbness, reflective of direct damage to the integrity of peripheral nerves. Severe cases may require either dose reduction or early termination of chemotherapy, decreasing the therapeutic effectiveness and potentially even decreasing life expectancy. Rodent models are employed to model PIPN both for mechanistic and pre-clinical therapeutic studies, but the pain research community has often voiced a skepticism of the utility of PIPN models, citing high variability in behavioral outcome measurements as a cause to seek other models of chemotherapy-mediated neuropathies. Here we report a simple, robust, reproducible PIPN protocol that is fast-acting, effective over the course of three weeks time after paclitaxel injections, and is efficacious in both males and female cohorts of both inbred and outbred mouse and rat strains. Mechanoceptive and thermoceptive behavioral outcomes were measured using standard von Frey, Hargreaves, and cold plate assays, respectively. We also observed substantially-different time courses of behavioral effects when directly comparing standard, commercially-available formulation of paclitaxel to a clinical formulation used in human patients. Pharmacokinetic studies are underway to explain the strong differences we observed in behavioral response to different formulations of paclitaxel.

Disclosures: D. Giuvelis: None. **I.D. Meng:** None. **K.L. Tucker:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.10/I15

Topic: D.03. Somatosensation – Pain

Title: Further investigation into the translational utility of oxaliplatin induced behavioural models

Authors: *A. S. FISHER, A.-L. THOMAS, C. MILLUM, N. UPTON;
Transpharmation, London, United Kingdom

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) and cognitive impairments e.g. “chemo-brain” are well known dose limiting side-effects of platinum based antineoplastic drugs e.g. oxaliplatin.
These studies aimed to extend our previous work on the global effects of oxaliplatin by investigating the consequences of systemic oxaliplatin in the novel object recognition (NOR) test; a model of spontaneous exploratory behaviour measuring recognition memory.

Additionally, we wanted to establish an acute model of intraplantar (i.pl.) oxaliplatin-induced peripheral neuropathy to enable pharmacodynamic assessment of novel chemical entities with improved throughput for drug discovery programmes.
Chemo-brain was induced by a single intraperitoneal (i.p.) injection of oxaliplatin (6 & 10mg/kg i.p.) to male Lister Hooded rats (200-250g at time of injection). Sham animals received 5% dextrose i.p. A deficit in mnemonic processing was evaluated using a 1 hr inter trial interval (ITI).
Chemotherapy-induced acute hypersensitivity was elicited in male C57Bl/6 mice (25-30g) by injection of oxaliplatin (20 & 30ug/20ul i.pl.). Spontaneous nocifensive behaviours were observed from 0-60 mins post injection. Mechanical (vonFrey, vF) and cold sensitivity (cold plate) were tested at 1h, 4h, 24h, 48h, 5 & 7 days post i.pl. The gold standard reference compounds Pregabalin (PGB, 30mg/kg p.o.) and Gabapentin (GBP, 60mg/kg i.p.) were tested for efficacy in this novel model.
Animals receiving a single administration of oxaliplatin (6 & 10mg/kg) demonstrated impaired recognition of a novel object following a 1h ITI as demonstrated by a significant reduction in the discrimination (D2) index; the most stringent NOR performance measure. No overt clinical signs were observed on the overall wellbeing and locomotion of the animals.
Acute chemotherapy induced peripheral neuropathy was successfully induced by local i.pl. injection of oxaliplatin dose-dependently. Spontaneous pain behaviours were significantly increased and both PGB and GBP reduced them by 76 & 93% respectively. Mechanical and cold sensitivity were rapidly induced and still present at 7 days of testing. Mechanical hypersensitivity was reduced 88 & 89% by PGB and GBP respectively. However, the acute cold allodynia was relatively untouched by these two α2-δ subunit inhibitors.
Local i.pl. injection of oxaliplatin represents a clinically translatable acute model of chemotherapy induced neuropathy. PGB and GBP reduced the mechanical sensitivity but had little-no effect on the thermal test. This differential effect of PGB and GBP on distinct thermal and mechanical modalities is currently being investigated.

Disclosures: **A.S. Fisher:** None. **A. Thomas:** None. **C. Millum:** None. **N. Upton:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.11/I16

Topic: D.03. Somatosensation – Pain

Support: Swiss SNSF n°310030_179169

Title: Glial cells activation in a mouse model of alcohol-induced neuropathic pain

Authors: *P. CHU SIN CHUNG, M. PERTIN, G. KIRSCHMANN, I. DECOSTERD; CHUV, Lausanne, Switzerland

Abstract: Introduction: Neuropathic pain affects millions of people worldwide and Alcohol Use Disorder (AUD) affects about 4% of the world population. Clinical observations emphasized the strong comorbidity between these two highly debilitating and chronic pathologies. The estimated prevalence of alcohol-related neuropathy is about 25-66% among who meet criteria for AUD, with an estimated 1/3 of painful neuropathy. The neurobiological mechanisms responsible for alcohol-induced neuropathic pain remain to be further elucidated. Glial cells have been described to be activated into the central nervous system, in neuropathic pain as well as AUD models, independently. We hypothesized that the glial cells activation would play an essential role in an alcohol-induced neuropathic pain condition.
Methods: In this study, a total of 75 males and females adult CX3CR1-eGFP reporter mice were used to specifically visualize the microglia cells. 38 mice undergo intermittent alcohol exposure model, a diet of ethanol 6.5% for 5 days a week, while 37 mice were exposed to a control water diet for the whole week. The mechanical and thermal sensitivity was assessed once a week by classical Von Frey and Hargreaves behavioral tests. After 10 weeks of intermittent alcohol exposure, the number of CX3CR1-eGFP and Glial fibrillary acidic protein (GFAP) positive cells from hypersensitive animals or controls were counted into the dorsal root ganglion (DRG) and the spinal cord (SC).
Results: We observed a significant decrease of the pain threshold within 4 weeks of intermittent alcohol exposure in the group exposed to alcohol in comparison with the control water group. Correlation analysis at different time points suggest that the high alcohol intake decreases the sensitivity at early stages but then is associated with an hypersensitive phenotype after a long-lasting exposure. The analysis of glial cells activation revealed statistical increase of microglia- and astrocyte-like cells after alcohol intoxication or no significant changes, respectively into the DRG or the SC.
Conclusion: Chronic alcohol consumption induces neuropathic pain and triggers microglia- and astrocytes-like cells activation, in the peripheral nervous system. Although future experiment will be required to precisely define the role of glial cells in alcohol-induced neuropathic pain, SGC represent potentially major actors of the neurobiological process involved in the comorbidity.

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Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.12/I17

Topic: D.03. Somatosensation – Pain

Title: Partial crush injury: A new experimental model for chronic neuropathic pain

Authors: *H. KIM[1], C. WON[1], W. KIM[1], S. CHUNG[1], S. OH[2];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Sch. of Dent, Seoul Nat'l Univ., Seoul, Korea, Republic of

Abstract: In our recent study (Davies *et al.*, 2019), we have shown that, after axonal reinnervation, higher mechanical hypersensitivity persists in mice with partial crush injury (PCI) in sciatic nerve rather than the mice with full crush injury. This implies that injured axons which remain after partial crush induce the chronic mechanical hypersensitivity. In this study, we aimed to further characterize PCI in adult mice, in comparison with full crush injury, to develop a new experimental model of neuropathic pain.
The crush injuries in sciatic nerve were produced by using ultra-fine hemostat with (for partial crush) or without foil gap (for full crush) from 7-8 weeks adult mice. To determine functional recovery of the sciatic nerve after surgery, we used foot print analysis method for motor function and pin prick test for sensory function. We also compared mechanical and thermal pain behaviors between two crush models, by using von Frey filaments and Hargreaves' test, respectively.
Both PCI and full crush groups took about 2 weeks to fully recover their motor and sensory function after surgery. The motor recovery showed similar pattern in both groups, whereas sensory function was dramatically different between two groups. Sensory function was not completely lost right after the PCI, showing higher pin prick score than the full crush at early time points. In PCI group, tactile hypersensitivity was induced after axonal reinnervation and maintained over 60 days. Thermal hypersensitivity was induced even before the reinnervation in mice with PCI, which lasted up to 21 days. In contrast, mice with full crush injury did not show mechanical or thermal hypersensitivity at any time points.
Taken together, our results suggest that difference in damaged fiber types following sciatic nerve crush leads to differential pain hypersensitivity between full crush and partial crush. We propose PCI as a novel chronic neuropathic pain model, potentially useful not only to dissect out molecular mechanisms for mechanical and thermal hypersensitivity, and but also to provide therapeutic target for the intervention of neuropathic pain.

Disclosures: H. Kim: None. **C. Won:** None. **W. Kim:** None. **S. Chung:** None. **S. Oh:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.13/I18

Topic: D.03. Somatosensation – Pain

Support: NIH NINDS: 1 R01 099338

Title: Characterization of bone remodeling and peripheral sprouting in the K/BxN male and female mouse

Authors: *G. G. DOS SANTOS[1], J. JIMENEZ-ANDRADE[3], E. MUÑOZ-ISLAS[3], M. B. RAMIREZ-ROSAS[3], G. F. CATROLI[1], N. OHASHI[1], T. L. YAKSH[1], M. CORR[2];
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Abstract: Background: Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by joint inflammation, cartilage destruction, and chronic pain. Sex is an important biological variable in the development and treatment of RA. In this work we examined the chronic arthritic phenotype in a chronic murine a model of arthritis in male and female mice.
Aim: The current set of experiments aimed to characterize the murine K/BxN model of arthritis in:1) Ankle and joint remodeling 2) Glial activation in spinal cord and 3) Sympathetic and afferent sprouting in the Dorsal Root Ganglia (DRG)
Methods: Male and female wild type (WT) C57BL/6 mice Harlan, Indianapolis, IN, KRN T cell receptor transgenic mice on the B6 background were a gift from Drs. D. Mathis and C. Benoist. Arthritic mice were obtained by crossing K/B with NOD/Lt (N) animals (K/BxN). Mechanical withdrawal thresholds (TA) were assessed in 8, 10, 12, 14 and 16 wk old mice (von Frey hair/up-down method). At 16 wk of age, legs, DRG, and spinal cords were harvested. Quantitative micro-CT was performed on limb/joints. Spinal cord (L4–L6) were immunostained for microglia (IBA1) and astrocytes (GFAP); DRG (L4-L5) were immunostained for neuron (NeuN), macrophage (IBA1), sensory fiber (CGRP), sympathetic (TH), and neuronal injury (ATF3) markers; ankle joints were immunostained for neuronal growth (GAP-43), CGRP, and TH.
Results: As compared to WT, males and female K/BxN mice showed a persistent mechanical allodynia (thresholds <0.5 g), prominent bone loss in the ankle joint´s bones; an increased IBA1 activation in dorsal horn while females showed increases in IBA1 and GFAP. Males and females showed increased TH and CGRP with no apparent ATF3 activation in the DRG. In ankle joint synovium, an increased density of TH, CGRP, and GAP-43 fibers was detected. TA was reversed transiently by gabapentin, but not by the NSAID ketorolac.
Discussion and conclusion: The K/BxN arthritic phenotype is characterized by i) a persistent tactile allodynia, ii) periarticular sympathetic sprouting iii) an upregulation of peptidergic afferent innervation in peripheral tissue and iv) activation of spinal microglia (IBA1) in male and astrocytes (GFAP) in females. The lack of effect of gabapentin vs ketorolac suggests a neuropathic profile which may reflect a peripheral phenotype compatible to a polyneuropathy.

Disclosures: G.G. Dos Santos: None. **J. Jimenez-Andrade:** None. **E. Muñoz-Islas:** None. **M.B. Ramirez-Rosas:** None. **G.F. Catroli:** None. **N. Ohashi:** None. **T.L. Yaksh:** None. **M. Corr:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

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Program #/Poster #: 054.14/I19

Topic: D.03. Somatosensation – Pain

Support: DOD W81XWH-17-1-0542 to CMP
DOD W81XWH-17-1-0541 to YS

Title: Characterization of a syngeneic mouse model of prostate cancer induced bone pain

Authors: R. M. CAIN[1], S. H. PARK[1], M. R. EBER[1], R. PARKER[1], J. M. JIMENEZ-ANDRADE[2], Y. SHIOZAWA[1], ***C. M. PETERS**[1];
[1]Wake Forest Med. Sch., Winston-Salem, NC; [2]Autonomous Univ. of Tamaulipas, Reynosa, Mexico

Abstract: Cancer-induced bone pain (CIBP) is the most common (75-90% of patients) and devastating symptom of bone metastatic cancer, and it substantially disrupts patient's quality of life. Currently, there are no effective analgesic treatments for CIBP other than opioids which come with severe side effects. In order to better understand the factors and mechanisms responsible for CIBP it is essential to have clinically relevant animal models that mirror pain related symptoms and disease progression observed clinically.
We recently characterized a syngeneic mouse model of prostate cancer induced bone pain which has the advantage of allowing studies in immunocompetent animals which better reflect the normal bone microenvironment. Male C57Bl6 mice were inoculated into the femur with RM-1 prostate cancer cells ($1x10^3$ cells/5µl) or Hank's buffered saline as a sham control. Prior to injection, the RM-1 prostate cancer line was transfected with luciferase reporters and green fluorescent protein (GFP) in order to visualize *in vivo* tumor growth longitudinally and quantify the extent of tumor burden at sacrifice. We observed a progressive increase in bioluminescence (BLI) in the ipsilateral hind limb of RM-1 but not sham mice between 7 and 21 days post-inoculation. Tumor growth was accompanied by spontaneous guarding of the inoculated limb suggestive of ongoing pain and impairment of daily running wheel performance indicative of movement evoked pain. Tumor induced bone remodeling, evident as cortical osteolytic lesions and extra-periosteal aberrant bone formation, was observed in the ipsilateral femur of RM-1 but not sham mice based on histological and radiographic analysis. The number of sensory (CGRP+, NF200+) nerve fibers was quantified in the periosteum, mineralized bone and bone marrow of tumor bearing and sham inoculated mice. Ectopic sprouting of sensory neurons was observed predominantly within the periosteum in close proximity to prostate cancer cells and regions of remodeled bone. Additionally, spinal cord tissue was collected and examined immunohistochemically for markers of central sensitization (pERK, dynorphin) and glial

activation (GFAP, IBA1).
Future studies will examine the interaction between sensory neurons, immune and tumor cells within the bone microenvironment and investigate cellular mechanisms by which sensory neuron derived factors contribute to prostate cancer induced bone pain, skeletal remodeling, and tumor progression.

Disclosures: R.M. Cain: None. **S.H. Park:** None. **M.R. Eber:** None. **R. Parker:** None. **J.M. Jimenez-Andrade:** None. **Y. Shiozawa:** None. **C.M. Peters:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: National Natural Science Foundation of China (NFSC#81671086)
SUSTech Foundation #Y01416102

Title: Characteristics of streptozotocin-induced diabetic neuropathic pain in rats and mice

Authors: *H. LIAO, J. HU, R. HUANG, X. ZHANG, X.-J. SONG;
SUSTech Ctr. for Pain Med. and Med. Sch., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Diabetic neuropathic pain (DNP) is one of the most common complications of diabetes mellitus. Mechanisms underlying DNP remain elusive and the clinical approaches for DNP treatment are limited. We have noticed that the two most commonly used experimental animal models of DNP induced by streptozotocin (STZ, i.p.) in Sprague-Dawley rats and C57BL/6 mice showing a large variation in DNP incidence, we thus aimed to further elucidate the characteristics of DNP in these models in order to provide more reliable and stable parameters for studying DNP. STZ-induced type 2 diabetes models were made in SD rats (single dose, 70 mg/kg) and C57BL/6 mice (40 mg/kg, daily for 5 consecutive days). The success model of diabetes was evaluated by the persistent high blood glucose (>16.6mmol/L for rats and >13.8mmol/L for mice) combined with the pancreas damage and peripheral nerve neuropathy. DNP was evaluated with an additional painful mechanical allodynia. Following STZ treatment, 87/96 (90.63%) SD rats developed high blood glucose. The significantly increased level of blood glucose started within 7 days and kept gradually increasing and maintained over the entire experiment period of 7 weeks. Of the rats exhibiting high blood glucose, 43/87 (49.43%) developed mechanical allodynia, while the rest diabetic rats did not exhibit mechanical allodynia and other obvious painful symptoms. In C57BL/6 mice, STZ-induced high blood glucose was seen in 41/54 (75.93%) animals. The level of blood glucose increased and peaked within 14 days

and the second overshot was seen at the testing point of 7th week. Of those hyperglycemic mice, 35/41(85.37%) exhibited mechanical allodynia, which started within 7 days and persisted over the entire experimental period to 7 weeks. Thermal hypersensitivity to radiant heat was not seen in these rats and mice. These findings demonstrate that the incidence of STZ-induced DNP in diabetic SD rats (~50%) is greatly lower than that in diabetic C57BL/6 mice (~85%). Considering together hyperglycemia and DNP, there are only approximately 45% (90.63%*49.43%) SD rats and 65% (75.93%*85.37%) C57BL/6 mice can be classified as DNP animals. Our preliminary clinical survey showed that there was 16.9% (11/65) patients with type 2 diabetes with certain painful experience. This study indicates that it is necessary to identify the behaviorally expressed painful symptoms in addition to detecting blood glucose and other signs of diabetes in each of the STZ-SD rats and STZ-C57BL/6 mice when we use them to study DNP.

Disclosures: H. Liao: None. **J. Hu:** None. **R. Huang:** None. **X. Zhang:** None. **X. Song:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.16/I21

Topic: D.03. Somatosensation – Pain

Support: DoD OR170276
Sheila and David Fuente Phantom Limb Neuropathic Pain Research Program

Title: Phantom limb pain model in rats: Behavioral and histochemical evaluation

Authors: *H. C. MARTINEZ[1], A. LANJEWAR[2], A. NIEDECKEN[1], C. MARCH[1], B. I. SCHACHNER[1], S. M. NOUDALI[1], M. NESHEIWAT[1], S. JERGOVA[1], J. SAGEN[1];
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Abstract: Phantom Limb Pain (PLP) is a common phenomenon in amputees, often reported as a severe pain. Therapeutic strategies targeting PLP provide inadequate pain relief, mostly due to complexity of PLP. Gene-based therapy is a tool for multitarget yet local interventions. The goal was to develop a preclinical PLP model in rats and to evaluate efficient delivery routes for gene-based therapy. Animal PLP models are based on deafferentation injury followed by autotomy. Clinical studies showed that the presence of pre-amputation pain increase the risk of developing PLP. We used a combination of chronic constriction injury (CCI) and formalin injections at various time points prior to axotomy to mimic clinical scenarios of the presence of pain prior to medically indicated amputation. Sprague Dawley males were used to induce PLP using sciatic nerve axotomy. Behavior was recorded and scored daily. Animals were perfused within two months and sciatic nerve, dorsal root ganglia (DRGs), spinal cord, brain and samples of cerebrospinal fluid (CSF) removed and processed for immunohistochemistry (NaVs, CGRP,

Substance P, c-Fos, GAD65/67, NK1, IBA-1, GFAP) and ELISA (IL1beta). Intraspinal, intrathecal or DRG injection of AAV8/GFP was used to detect the most efficient route for the delivery of therapeutic genes. Results show development of autotomy reflecting the presence and timing of the pre-amputation injury. The presence of pain prior to axotomy resulted in more extensive autotomy compared to behavior induced by axotomy only. Animals with CCI displayed more rapid autotomy onset when axotomy occurred either shortly after CCI (1 day after) or at 4 weeks post CCI. Delayed autotomy onset was observed when axotomy was done at 1 week post CCI. Elevated levels of IL-1beta were detected in CSF of animals with autotomy compared to non-responders (same injury but no autotomy). Immunohistochemical analysis showed enhanced expression of NaV 1.7 in DRGs and spinal cord in rats displaying autotomy. Ipsilateral sprouting of CGRP fibers into deeper spinal laminae and enhanced expression of NK1 receptor was observed primarily in animals with robust PLP behavior. GFP was localized within the superficial and deep dorsal horn near the injection sites after intra-spinal injection of AAV8/GFP. Intrathecal delivery showed superficial labeling with modest penetration to the dorsal spinal gray matter. Intra-DRG injection resulted in labeling of processes and cell bodies in superficial laminae and in DRG neurons. Our results show that this model may be useful in evaluating novel treatment strategies for PLP and suggest possible neurochemical targets for therapy.

Disclosures: H.C. Martinez: None. **A. Lanjewar:** None. **A. Niedecken:** None. **C. March:** None. **B.I. Schachner:** None. **S.M. Noudali:** None. **M. Nesheiwat:** None. **S. Jergova:** None. **J. Sagen:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.17/I22

Topic: D.03. Somatosensation – Pain

Title: Pain-induced impulsivity in rats and its reversal by various agents

Authors: *S. G. SAPUTRA, N. ESPINOZA, A. NAZARIAN;
Pharmaceut. Sci., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Pain sensation is a complex process that consists of sensory, affective and cognitive components. The focus of this project is to examine the cognitive component of pain, which consists of various corticolimbic processes including impulsivity. Examination of pain-induced impulsivity is critical due to the association of impulsivity with the development of psychiatric comorbidities observed in chronic pain patients, including depression, anxiety, and substance use disorders. One determinant sign of impulsivity is the devaluation of future rewards of large magnitude in favor of obtaining immediate rewards of small magnitude (i.e. impulsive choice).

The goal of this project is to determine if opioid and non-opioid drugs reduce pain-induced impulsivity in male and female rats.
To measure impulsive choice, animals were trained in the delay discounting task for food pellets using a delay schedule of 0, 8, 16, and 32 seconds. After training was complete and baseline measurements were established, rats received intraplantar Complete Freund's Adjuvant (CFA). Animals were then assigned to the delay discounting task and the effects of opioid and non-opioid agents were examined again.
Our preliminary findings showed that morphine treatments decreased pain-induced impulsivity in male and female rats. However the effect of morphine gradually diminished as the treatment continued and eventually became ineffective during the two-weeks testing period, indicating the development of tolerance. As pain is once central component that drives impulsivity, we seek to determine if non-opioid drugs, such as nonsteroidal anti-inflammatory drug (NSAID) and drugs used for the treatment of impulsivity could block pain-induced impulsivity. Results of these studies will be reported.

Disclosures: **S.G. Saputra:** None. **N. Espinoza:** None. **A. Nazarian:** None.

Poster

054. Pain: Animal Models of Behavior

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Topic: D.03. Somatosensation – Pain

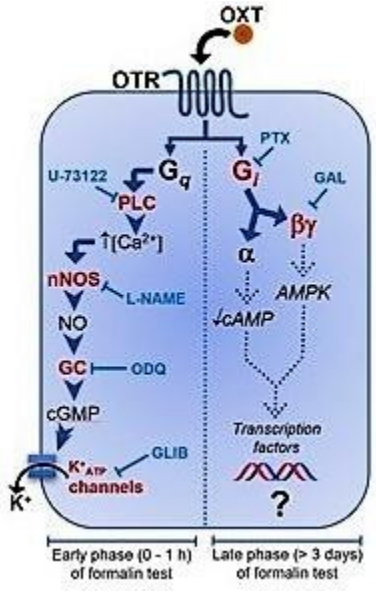
Support: PAPIIT-UNAM Grant IN200415 (MCL)
PAPIIT-UNAM Grant IN203117 (AGH)
PAPIIT-UNAM Grant IN203119 (AGH)

Title: Spinal oxytocin inhibits early and late formalin-induced nociception by recruitment of two differential intracellular pathways

Authors: *A. GONZALEZ-HERNANDEZ, A. DE LOS MONTEROS-ZÚÑIGA, G. MARTINEZ-LORENZANA, M. CONDES-LARA;
Neurobiología del Desarrollo y Neurofisiología, Inst. de Neurobiologia, UNAM, Queretaro, Mexico

Abstract: Oxytocin (OXT) has described as a mediator of endogenous analgesia. Indeed, oxytocinergic projection from the hypothalamic paraventricular nuclei (PVN) to the superficial laminae of the spinal dorsal horn exists and intrathecal oxytocin relieves pain in humans under pathological conditions. Notwithstanding, the precise spinal in vivo mechanism underlying this effect is not well identified. Using male Wistar rats the effects of spinal OXT (10 nmol) administration on the pain behavior was evaluated. Spinal OXT (10 nmol) or PVN electrical

stimulation (6-sec at 60 Hz with a pulse duration of 1-msec and 300 mA) diminished the early (1-h; measured as paw flinches) and late (3-30 days; measured as 50% withdrawal threshold) nociception induced by formalin (1%).
The early OXT antinociceptive effect was: (i) reversed by L-368,899 (10 nmol; OTR antagonist), U-37122 (4 nmol; inhibitor of phospholipase C, PLC), L-NAME (370 nmol; inhibitor of nitric oxide synthase, NOs), ODQ (50 nmol; inhibitor of nitric oxide-sensitive guanylyl cyclase, GC) or glibenclamide (10 nmol; and ATP-sensitive K channel blocker, K_{ATP}-channel); and (ii) unaffected by pertussis toxin (PTX, 1µg/rat; an inhibitor of G-protein signaling) or gallein (137 nmol an inhibitor of G protein βγ subunit-dependent signaling).Interestingly, OXT-induced inhibition of late nociception was not only blocked by the L-368,899, but also by PTX or gallein. In contrast, spinal pre-treatment with U-37122 or L-NAME did not any effect on the OXT late effect. All drugs were given by an intrathecal injection in a volume of 10 ul. The OXT was given 10-min prior to formalin injection (50 ul, injected s.c. into the dorsal surface of the right hindpaw). PTX pre-treatment was performed 1 week before the formalin test. All antagonists and blockers were given 10-min prior OXT treatment
Taken together, the above findings suggest that spinal OXT-induced antinociception could be mediated by two possible differential intracellular pathways, in both cases dependent on the OTR activation.



Disclosures: A. Gonzalez-Hernandez: None. **A. de los Monteros-Zúñiga:** None. **G. Martinez-Lorenzana:** None. **M. Condes-lara:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant AR061371
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Helen C. Levitt Award from U Iowa COM

Title: Testosterone and resistance exercise training protect against the development of chronic widespread muscle pain

Authors: *J. LESNAK[1], S. INOUE[1], L. RASMUSSEN[1], K. A. SLUKA[2];
[2]Physical Therapy & Rehab Sci., [1]Univ. of Iowa, Iowa City, IA

Abstract: Chronic widespread pain conditions are more common in women, with a greater female presentation of movement-related pain suggesting sexual differences in the developmental mechanisms of widespread pain. Female mice develop bilateral, more severe and longer duration hyperalgesia compared to male mice in a model of activity-induced muscle pain. We examined if testosterone protects against the development of widespread fatigue-induced muscle pain. Muscle hyperalgesia was induced by two sub-threshold muscle insults (20μl normal saline, pH 5.0, 5 days apart) and fatiguing muscle contractions (6 min electrically-induced contractions) in C57BL6/J mice (n=97). Hyperalgesia was assessed as decreases in muscle withdrawal thresholds to pressure applied over the gastrocnemius muscle by force-sensitive tweezers. We examined if 1) orchiectomy, 2 weeks before induction of the model, prevented the development of bilateral and long-lasting hyperalgesia, when compared to intact male and female mice, and 2) 2 weeks of testosterone administration reversed effects of orchiectomy in males and reduced widespread hyperalgesia in females. We examined 3 conditions 1) muscle insult and fatigue given at the same time in the same muscle, 2) muscle insult and fatigue given at the same time in opposite muscles, and 3) muscle fatigue given 24h before muscle insult. Female mice developed bilateral hyperalgesia in all 3 conditions, which was longer lasting than males. Male mice only developed unilateral hyperalgesia when the muscle insult and fatigue were given in the same muscle at the same time. In contrast, orchiectomized male mice developed bilateral hyperalgesia in all 3 conditions, and the hyperalgesia was longer-lasting, similar to that observed in female mice. Testosterone in female and orchiectomized male mice, shortened the hyperalgesia similar to that observed in intact male mice. A resistance training protocol also prevented the development of activity-induced hyperalgesia in male and female mice, which was reversed through the administration of the androgen receptor blocker flutamide.

These results suggest that testosterone and resistance training protect male and female mice from developing long-lasting widespread mechanical hyperalgesia in a model of chronic muscle pain.

Disclosures: **J. Lesnak:** None. **S. Inoue:** None. **L. Rasmussen:** None. **K.A. Sluka:** None.

Poster

054. Pain: Animal Models of Behavior

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Program #/Poster #: 054.20/I25

Topic: D.03. Somatosensation – Pain

Support: NIH Grant AG053783
NIH Grant DE027808

Title: Age- and sex-related differences in acute pain, osteoarthritis (OA)-like pain, and descending noxious inhibitory control (DNIC) in rats

Authors: ***J. Y. RO**[1], Y. ZHANG[1], C. TRICOU[2], J. T. DA SILVA[3];
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Abstract: Chronic pain, a significant healthcare issue, becomes more prevalent with age, and the management of pain conditions is extremely challenging in the elderly population. Chronic pain conditions, such as OA, that show increased prevalence with age also show prominent sex differences. However, there is only limited information on how sex and age intersect for nociceptive processing and how such interactions lead to more profound chronic pain conditions in females in different stages of life. In this study, we investigated age- and sex-related differences in acute and chronic pain conditions as well as in endogenous pain modulation in rats. Groups of male and female Fischer 344 rats consisting of both young (3-6 mo) and aged (20-24 mo) rats were used to assess basal thermal and mechanical thresholds. Capsaicin-induced acute nocifensive responses, c-Fos expression in the spinal cord dorsal horn, and monoiodoacetate (MIA)-induced knee OA-like pain responses were compared between both sexes and age groups. DNIC was also assessed with capsaicin in the forepaw as the conditioned stimulus and noxious heat on the hindpaw as the test stimulus. There was a significant sex, but not age, effect on basal noxious thermal thresholds and mechanical thresholds on the knee joint. No significant age- and sex-related differences in capsaicin-induced acute nocifensive responses and c-Fos expression levels in the spinal cord were observed. In all age and sex groups, MIA injection into the knee joint resulted in the development of significant mechanical hyperalgesia, as assessed by weight bearing responses (WBR). Aged male rats developed a greater peak reduction of WBR than young rats. Aged female rats developed the most profound weight bearing deficit. When mechanical sensitivity of the knee joint was used as an outcome measure,

MIA induced more pronounced and longer- lasting hyperalgesia in older rats, with aged female rats showing greater hyperalgesia. Young males exhibited stronger DNIC responses than young females. DNIC responses were significantly impaired in both old male and old female rats. These data suggest that age does not have significant effect on basal and acute nociceptive processing, but it does significantly impact OA-like pain responses and DNIC, making aged rats, especially females, more vulnerable to chronic pain conditions. These preclinical models should provide important tools to further investigate basic mechanisms underlying the impact of age and sex in chronic pain conditions.

Disclosures: **J.Y. Ro:** None. **Y. Zhang:** None. **C. Tricou:** None. **J.T. Da Silva:** None.

Poster

054. Pain: Animal Models of Behavior

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.21/I26

Topic: D.03. Somatosensation – Pain

Support: DHA Grant MR 190013

Title: Behavioral characterization of comorbid symptoms of pain, depression, and anxiety in thermally injured male and female rats

Authors: *B. CHEPPUDIRA, A. MARES, M. M. STRAIN, A. V. TREVINO, R. J. CHRISTY, S. L. CRIMMINS;
US Army Inst. of Surgical Res., San Antonio, TX

Abstract: Background: It is evident that burn injury and treatment procedures such as wound cleaning and debridement induce pain, depression, and anxiety in patients. Uncontrolled pain and associated psychological symptoms can lead to long-term physical and psychological disabilities. The mechanism of pain, depression, and anxiety comorbidity that often arises from a burn injury is not fully understood. One reason for this knowledge gap is due to the lack of a reliable animal model with clinically relevant comorbid symptoms of burn pain and depression. The objective of the present study is to characterize the comorbid symptom of depression and anxiety in male and female rats with thermal pain. **Method:** Thermal injury was induced in anesthetized adult male and female Sprague-Dawley rats by placing a pre-heated (100 °C) metal probe for 30 sec on the right hind paw. Comorbid symptoms (pain, depression, and anxiety) in thermally injured rats were measured at multiple time points. Mechanical and thermal allodynia was assessed using the von Frey and Hargreaves' tests. Depressive-like behavior was measured by the tail suspension test (TST). Anxiety-like behaviors were assessed using the open field and light and dark box assays. **Results:** Here we present the initial data collected between post-injury days 7 and 8. Thermal injury did induce mechanical and thermal allodynia in both male and female rats.

However, there was no significant difference in either mechanical or thermal allodynia between the sexes. Thermally injured male rats showed two-fold increases in immobility behavior than female rats in TST. In the open field test, thermally injured male rats displayed a significant reduction in exploratory behavior and also in the number of visits to the central arena compared to female rats with thermal injury. Male rats with thermal injury made less transition to and spent less time in the light compartment compared to female rats in light and dark box assay. **Conclusion:** Under our experimental condition, thermal injury appears to induce more intense depressive- and anxiety-like behaviors in male rats than in female rats but with a similar intensity of mechanical and thermal allodynia.

Disclosures: B. Cheppudira: None. **A. Mares:** None. **M.M. Strain:** None. **A.V. Trevino:** None. **R.J. Christy:** None. **S.L. Crimmins:** None.

Poster

054. Pain: Animal Models of Behavior

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Program #/Poster #: 054.22/I27

Topic: D.03. Somatosensation – Pain

Support: CCDRN/EU Norte-69-2015-15
Luso-American Foundation for Development

Title: Pain in osteoarthritis - Characterizing and predicting pain persistence after joint replacement

Authors: *J. BARROSO[1], K. WAKAIZUMI[2], A. VIGOTSKY[3], S. HUANG[3], T. SCHNITZER[3], V. M. GALHARDO[4], V. APKARIAN[3];
[1]Northwestern Universuty, Chicago, IL; [2]Physical Med. and Rehabil., Northwestern Univ., Feinberg Sch. of Med., Chicago, IL; [3]Northwestern Univ., Chicago, IL; [4]Faculdade Medicina Univ. Porto, Porto, Portugal

Abstract: Osteoarthritis (OA) represents a major cause of chronic musculoskeletal pain worldwide. Cumulative data suggests that in addition to nociceptive mechanisms elicited by the articular damage, disruption in central pain processing mechanisms may influence OA pain and its persistence after total joint replacement (TJR) surgery. Behavioral predictors of OA pain and post-surgery pain relief remain relatively under explored. Here, we assess OA pain characteristics in knee and hip patients and study its association with pain and pain relief after TJR. We followed knee and hip OA patients before, three and six months post-TJR. Measures of pain intensity and quality, behavior, mood and quality of life were acquired using multiple questionnaires, together with physical performance-based tasks assessed at all time points. Brain MRI scans were acquired 3-6 weeks before surgery. Baseline radiographic assessment of the

articular damage was also performed. Four different pain intensity outcomes were selected to characterize and to predict post-TJR pain. Multi-factorial regression models were tested, and a network analysis was applied to pain related biopsychosocial measures. A total of 84 knee, 22 hip OA patients and 36 control participants completed the study. We observed pain intensity for both OA groups was similar pre-surgically and hip OA patients presented greater relief than knee OA after surgery. Pain levels remained constant from 3 to 6 months after surgery in both groups. Pre-surgical pain levels were not associated to post-surgical pain and hierarchical multi-factorial regression models failed to reliably predict residual pain after surgery from clinical and behavioral measures (adjusted R^2:0.09-0.11). Network analysis of pain related biopsychosocial measures showed significant changes post-surgery in both groups with network topological properties (modularity, clustering coefficient), changing only in hip OA. Our results support the failure of pre-operative pain as a predictor of post-surgical outcomes and reveal the unpredictability of post-surgical OA pain based on pre-surgical clinical and psychological related parameters, although a significant reorganization of their interrelationships takes place after surgery. Brain morphometric properties in OA and its association with clinical pain properties are currently under investigation.

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Poster

054. Pain: Animal Models of Behavior

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Support: NIH COBRE award P20GM103643
NIH grant DK095143

Title: Osteoporosis-induced anxiety but not back pain is altered by chronic risperidone treatment

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Abstract: Osteoporosis is often associated with trabecular bone loss and compression fractures in the vertebral column leading to back pain. Risperidone administration across 8 weeks was shown to enhance trabecular bone loss in mice. We examined the hypothesis that Risperidone enhances OVX-induced vertebral bone loss and associated low back pain. Female mice (C57bl/6) underwent sham or OVX surgery at 5-weeks old. Starting 26 days post-OVX, daily Risperidone (0.75 mg/kg, p.o.) or equivolume saline was given daily across 8 weeks. Back pain was measured using grip strength before drug, during risperidone treatment and 7 days after

termination of drug treatment. Ongoing pain was assessed after risperidone treatment was terminated using conditioned place preference. Movement and rearing behaviors were assessed both during and after drug administration. OVX treated mice showed diminished grip strength compared to shams irrespective of Risperidone treatment. DAMGO reversed OVX-induced diminished grip strength but failed to induce conditioned place preference in any treatment group indicating that OVX induced evoked back pain but not ongoing pain. Analysis of open field movement and rearing revealed that OVX treated mice show diminished movement and rearing within 42 days post-OVX that persisted through 72 days post-OVX. Further analysis of movement revealed that OVX treated mice failed to show differences in time spent in the margin but had reduced center entries with 42 days post-OVX indicating a potential anxiety phenotype. Risperidone decreased center entries in both sham and OVX treated groups within 17 days of daily drug treatment. Testing 2-weeks post-treatment eliminated the drug-induced changes in center entries between Risperidone and vehicle treated mice in both sham and OVX groups. This reversal of Risperidone-induced changes in movement and behavior indicate that Risperidone may have anxiogenic effects independent of OVX status, and may enhance OVX-induced anxiety. In conclusion, OVX induces signs of back pain and anxiety. Chronic treatment with Risperidone at a dose demonstrated to enhance OVX-induced trabecular bone loss did not alter grip strength or DAMGO-induced CPP indicating that the Risperidone treatment fails to alter OVX-induced back pain. In addition, OVX mice demonstrated signs of anxiety within 42-days post-OVX, and Risperidone induced signs of anxiety within 17 days of treatment indicating that Risperidone may enhance OVX-induced anxiety. This research was supported by a faculty minigrant from the Office of Research and Scholarship at the University of New England, a COBRE award (P20GM103643) and an NIH grant DK095143 to KLH.

Disclosures: **A. Elkinson:** None. **V.E. Eaton:** None. **K.L. Houseknecht:** None. **T.E. King:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.24/I29

Topic: D.03. Somatosensation – Pain

Support: NIH GRANT DE026807

Title: Behavioral pain assessment in a dental pulp injury model

Authors: *H. L. ROSSI[1], L. P. SEE[2], C. H. MITCHELL[4], I. ABDUS-SABOOR[3]; [2]Endodontics, [3]Neurosci., [1]Univ. of Pennsylvania, Philadelphia, PA; [4]Univ. Pennsylvania, Philadelphia, PA

Abstract: Tooth inflammation produces the most excruciating pain ever experienced, yet very little is known about the molecular identities of tooth pulp afferents responsible for transmitting this pain. Transgenic mice that permit specific manipulation of tooth pulp afferents are one tool we may use to address this gap in our knowledge, but very little work has been done to characterize behavioral measures of dental pain in the mouse. We sought to address this issue by developing our own approach to facial Von Frey based on the existing literature and using the Mouse Grimace Scale to evaluate nociception before and after tooth pulp exposure. For Von Frey, mice were placed in chambers with an adjustable opening. The mice naturally put their faces out of this opening, which allows the experimenter to stimulate each side of the face twice with a graded series of Von Frey filaments. Each response to stimulation is scored from 0 (no response) to 4 (withdrawal followed by more than 2 facial wipes) according to the work of Vos et al. 1994. Threshold was considered any filament that scores 3 (withdrawal and 2 wipes, or attacking/biting the filament), or the point at which the mouse no longer voluntarily passes its face out of the opening for stimulation. For Grimace, we recorded the mice in a small enclosed chamber for 10 minutes and extracted 10 clear facial images for each video. Images were randomized and scored 0, 1, or 2 in treatment and day-blinded manner for the 5 action units described by Langston et al. 2009. Following baseline behavior, we anesthetized the mice and drilled the enamel and dentin to expose the pulp of the left first maxillary molar. Sham treatment consisted of the same anesthesia and manipulation without drilling (n = 6/treatment). Mice with pulp exposure had significantly greater Von Frey scores beginning on day 4, and a significant lower thresholds versus shams on day 6 post-exposure, on both sides. Grimace scores were significantly greater in mice with pulp exposure at all post-procedural time points, as compared both to baseline and shams. This indicates that pulp exposure can produce measureable indicators of nociception. Future work will evaluate changes in expression of labeled afferent lines to determine the most appropriate targets to alleviate pain related to pulp exposure.

Disclosures: H.L. Rossi: None. **L.P. See:** None. **C.H. Mitchell:** None. **I. Abdus-Saboor:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.25/I30

Topic: D.03. Somatosensation – Pain

Support: Conacyt, Numero de beca 492300

Title: Standardization of the up down behavioral model for the evaluation of tactile allodynia and mechanical hyperalgesia induced by traumatic spinal cord injury in Wistar rats

Authors: *A. MATA-BERMUDEZ[1,2], C. RÍOS-CASTAÑEDA[3,2], A. DIAZ-RUIZ[2];
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Abstract: Neuropathic pain is characterized as a chronic condition that arises as a result of injury or dysfunction in the somatosensory nervous system with complex alterations in cognitive and emotional functions. Causes of neuropathic pain commonly include a variety of conditions, such as viral infections, tumors, metabolic disorders and damage to the central or peripheral nervous system. Traumatic lesion of the spinal cord produces a painful sensation, representing a frequent complication that further deteriorates the quality of life of patients suffering from this disease, with various clinical manifestations such as musculoskeletal pain, radicular pain and visceral pain. The pain derived from the spinal cord injury has been underestimated for a long time, especially the neuropathic pain that has been cataloged as an important factor in the decrease of the quality of life and has been shown to produce adverse impact on a great variety of daily activities. The present project was aimed to evaluate the development and maintenance of tactile allodynia and mechanical hyperalgesia induced by spinal cord injury. Wistar female rats were used, with an approximate weight of 200g to 250 g (n=6). After surgical preparation and exposure of the dorsal vertebral column, the animals were dropped a metal cylinder of 10 g of weight at a height of 6.25 mm (T10) and at a height of 6.25 mm and 12.5 mm (T12) in order to produce tactile allodynia and mechanical hyperalgesia, which is a characteristic behavior of neuropathic pain. Tactile allodynia and mechanical hyperalgesia were evaluated by measuring paw withdrawal in response to probing with a series of calibrated von Frey filaments. The strength of the von Frey stimuli ranged from 0.4 to 15 g. Withdrawal threshold was tested by increasing and decreasing stimulus strength eliciting paw withdrawal. Traumatic lesion of the spinal cord produced tactile allodynia and mechanical hyperalgesia in female rats at vertebrae T12 (6.25 mm and 12.5 mm) level which was noticed by a diminished 50% paw withdrawal threshold that was less than (4 g) in the ipsilateral and contralateral paw but this effect is not observed in those who were injured in T10 compared with the sham group (15 g). Tactile allodynia was observed from Days 17 to 30. To our knowledge, standardization of the neuropathic pain model in Wistar rats with traumatic lesion of the spinal cord has not been reported, however, our work shows that tactile allodynia and mechanical hyperalgesia is produced when T12 is injured at two different intensities and such an effect was not observed on traumatic lesion of T10.

Disclosures: A. Mata-Bermudez: None. **C. Ríos-Castañeda:** None. **A. Diaz-Ruiz:** None.

Poster

054. Pain: Animal Models of Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 054.26/I31

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS095057
State of Washington Initiative Measure No. 171

Title: Tetrahydrocannabinol enhances spontaneous pain in a rat model of inflammatory bowel disease

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Abstract: Inflammatory Bowel Disease (IBD) is a lifelong condition that often begins between the ages of 15 and 30 and includes chronic pain as a major symptom. Treatment options are limited because NSAIDs and opioids have adverse effects. Although anecdotal reports suggest cannabinoids may be an effective treatment, few experimental studies have been conducted. Although animal models of IBD have been developed, assessment of spontaneous pain from the bowel is difficult to assess in animals. The two goals of this study were to determine: A) Whether home cage wheel running is an effective method to assess spontaneous pain caused by IBD, and B) Whether THC, the primary psychoactive compound in cannabis, can reverse this pain and restore wheel running. Adult and adolescent female Sprague-Dawley rats were habituated to the running wheel by placing each rat in a cage with a running wheel for 7 days. The number of wheel revolutions was assessed 23 hours a day. Immediately prior to the beginning of the dark phase on Day 8, rats were injected with trinitrobenzene sulphonic acid (TNBS) into the rectum to induce IBD-like symptoms. One day later, both vehicle and TNBS treated rats were injected with a low dose of THC (0.32 mg/kg, s.c.) or vehicle. Administration of TNBS depressed wheel running in adolescent rats for approximately 3 days and in adult rats for 5 days. Administration of THC did not alter wheel running relative to vehicle treated control rats during the 23 hours following administration in normal or TNBS treated adolescent or adult rats. However, this single injection of THC prolonged TNBS-induced depression of wheel running for over 5 days in both adolescent and adult rats. These data show that TNBS-induced IBD depresses wheel running as we have reported previously in rats with other pain conditions (i.e., hindpaw inflammation, migraine-like pain, and corneal abrasion) (Kandasamy et al., 2016; 2017; Hegarty et al., 2018). Unlike previous research, THC did not produce antinociception, but prolonged the negative effects of IBD.

Disclosures: J. Dunford: None. **A.T. Lee:** None. **M.M. Morgan:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.01/I32

Topic: D.03. Somatosensation – Pain

Title: Transient receptor potential vanilloid 4 ion channel participates in C-fibers but not in ADelta-fibers in mechanonociception of the normal and inflamed joint

Authors: *F. RICHTER, G. SEGOND VON BANCHET, H.-G. SCHAIBLE;
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Abstract: The Transient Receptor Potential vanilloid 4 ion channel (TRPV4) is an important sensor for osmotic and mechanical stimuli in the musculoskeletal system, and it is also involved in processes of nociception. In this study we investigated the putative role of TRPV4 ion channels in joint pain. Healthy adult WISTAR rats were anesthetized with sodium thiopentone (100 mg/kg, i.p.). The knee joint was mechanically stimulated by innocuous (20 mNm) or noxious (40 mNm) rotations of the lower leg against the fastened femoral bone for 15 s each. Action potentials were recorded from nerve fibers that were classified as C- or as Aδ-fibers by their conduction velocity (<1.4 m/s or <10 m/s, respectively). The TRPV4 antagonist RN-1734 and the TRPV4 agonists RN-1747, 4αPDD, and GSK 1016790A were tested. Compounds were injected into the joint cleft at a volume of 0.1 ml each. Acute joint inflammation was induced by injection of kaolin/carrageenan into the joint cleft and nerve fiber recordings were performed 7 hours after induction of inflammation. The intraarticular injection of the TRPV4 antagonist RN-1734 at 500 µM into the knee joint reduced the responses of C-fibers of the normal joint to noxious mechanical stimulation (control 214±55 APs/15s, after 3 hours 53±30 APs/15 s, respectively) and the responses of the sensitized C-fibers of the acutely inflamed joint to innocuous (control 127±28 APs/15s, after 3 hours 14±8 APs/15 s) and noxious (control 219±30 APs/15s, after 3 hours 74±42 APs/15 s) mechanical stimulation. In line with this the local mechanical thresholds in the receptive fields increased. The responses of nociceptive A∂-fibers were not significantly altered by RN-1734. The lower dose of 20 µM RN-1734 had similar effects on C- and A∂-fibers as the higher one. The intraarticular application of the TRPV4 agonists 4αPDD, GSK 1016790A, and RN-1747 did not consistently alter the responses of A∂- and C-fibers to mechanical stimulation of the joint nor did they induce ongoing activity. We conclude that TRPV4 ion channels are involved in the responses of C-fibers to noxious mechanical stimulation of the normal joint, and in the enhanced sensitivity of C-fibers to mechanical stimulation of the joint during inflammation of the joint.

Disclosures: F. Richter: None. **G. Segond von Banchet:** None. **H. Schaible:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.02/I33

Topic: D.03. Somatosensation – Pain

Title: TRPV1 antagonist BCTC inhibits pH 6.0-induced pain in human skin

Authors: *S. HEBER, G. HARTNER, C. I. CIOTU, M. GOLD-BINDER, N. NINIDZE, A. GLEISS, H.-G. KRESS, M. J. FISCHER;
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Abstract: Tissue acidosis due to relative and absolute ischemia occurs under several pathological conditions and is thought to contribute to pain in these conditions. TRPV1, TRPA1 and ASICs are known to be sensitive to acidic pH. In our previous approach, respective antagonists BCTC, A-967079 and amiloride were tested separately, but not in combination. Addressing potential interactions, these substances were injected in the volar forearm skin in 32 healthy volunteers in a pre-randomized, double-blind and balanced design. The present approach included an optimized three-step pH protocol which is closer to human pathophysiology and a full factorial design. Confirming the primary study hypothesis, the combination of all antagonists reduced acid-induced pain at pH 6.0. We observed an interaction of A-967079 with BCTC. BCTC inhibited pH 6.0-induced pain in the presence or absence of A-967079. Amiloride did not interact with other treatments and had no effect on acid-induced pain. The present study differs from the previous finding, which might be explained by the more relevant pH level. Inhibiting acidosis-induced pain could be a symptomatic and potentially also a disease-modifying approach.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.03/I34

Topic: D.03. Somatosensation – Pain

Support: R01NS087033

Title: Orai1 is required for metabotropic glutamate receptor 5-mediated calcium signaling

Authors: *H. HU[1], J. XIA[2], Y. DOU[4], Y. MEI[5], F. M. MUNOZ[3], D. LI[6];
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Abstract: Metabotropic glutamate receptor 5 (mGluR5) signaling has been implicated in many CNS diseases including pain. It is well known that activation of mGluR1/5 results in production of Inositol triphosphate (IP_3) and diacylglycerol (DAG), which lead to ERK activation. However, what are their signaling partners in dorsal horn neurons remain unclear. We have reported that store-operated calcium channels (SOCs), composed of STIM1/2 (Ca2+ sensors) and Orai1/2/3 (pore forming subunits) are expressed in dorsal horn neurons. Our recent study has demonstrated that Orai1 is responsible for SOC entry (SOCE) and plays a role in central sensitization associated with inflammatory pain, which raises an intriguing question: how Orai1 contributes to pain plasticity? Here, we tested the hypothesis that mGluR5 recruits Orai1 as part of its signaling pathway in dorsal horn neurons. We performed live-cell imaging in STIM1-YFP transfected dorsal horn neurons using time-lapse confocal microscopy and observed that neurotransmitter glutamate induced STIM1 puncta formation, which was not mediated by NMDA and AMPA receptors. Activation of mGluR1/5 by 100 μM Dihydroxyphenylglycine (DHPG) induced an initial transient calcium response, followed by a sustained phase in ~83% of total neurons, which was solely blocked by the selective antagonist of mGluR5, not mGluR1. Pretreatment with 3μM YM-58483 (a SOCE inhibitor) had no effect on the initial calcium response, however, almost completely blocked DHPG-induced the sustained calcium response and currents. Such effects were confirmed using Orai1 knockout (KO) neurons, suggesting that Orai1 is a key component mediating DHPG-induced calcium entry. We also found that DHPG-induced phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), modulation of A-type potassium channels and neuronal excitability were eliminated in Orai1 KO neurons. Consistently, our *in vivo* study showed that DHPG-induced nociceptive behavior and ERK activation are markedly reduced in Orai1 KO mice. These findings reveal that Orai1 is recruited in the mGluR5 signaling pathway and provide a novel molecular mechanism underlying mGluR5-mediated nociception, supporting a model in which activation of mGluR5 triggers Orai1 open, leading to an increase in p-ERK and modulation of neuronal excitability and nociception.

Disclosures: H. Hu: None. **J. Xia:** None. **Y. Dou:** None. **Y. Mei:** None. **F.M. Munoz:** None. **D. Li:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.04/I35

Topic: D.03. Somatosensation – Pain

Support: RO1NS087033

Title: Peripheral mechanism of store-operated calcium channel Orai1 in inflammatory pain

Authors: *Y. MEI[1], D. WEI[2], H. HU[3];
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Abstract: Chronic inflammatory pain is a substantial problem and is often managed improperly. Peripheral sensitization is one of the primary mechanisms underlying the pathogenesis of chronic inflammatory pain. However, candidate molecules involved in peripheral sensitization remain unclear. Our previous study showed that store-operated calcium channel pore subunit Orai1 plays an important role in formalin- and carrageenan-induced pain hypersensitivity. We have also reported that Orai1 is involved in central sensitization by modulating A-type potassium channels. Whether and how Orai1 contributes to peripheral sensitization associated with inflammatory pain is elusive. We have reported that Orai1 is functionally expressed in the peripheral nervous system in the dorsal root ganglion (DRG). To explore the peripheral mechanism of Orai1 in inflammatory pain, we performed patch clamp and Ca2+ imaging recordings in Orai1+/+ and Orai1-/- DRG neurons. We observed that thapsigargin (TG, an ER Ca2+-ATPase inhibitor)-induced SOC entry (SOCE) and SOC currents were significantly increased in Orai1+/+ DRG neurons, but not in Orai1-/- neurons after CFA or carrageenan injection. Interestingly, the expression level of Orai1 in DRGs was not altered, suggesting that the increased SOCE in DRG neurons is attributed to functional change of Orai1. To understand how Orai1 function is modulated under inflammatory pain conditions, prostaglandin E2 (PGE2), a prototype of inflammatory mediator, was used to sensitize DRG neurons. We found that TG-induced SOCE in DRG neurons was potentiated by PGE2, which was blocked by PGE2 receptor 1 (EP1) antagonists and PKC selective inhibitors, indicating PGE2-induced SOCE enhancement is mediated by EP1 and its downstream Gq-PKC cascade. Strikingly, PGE2-induced increase in neuronal excitability was markedly attenuated in Orai1-/- DRG neurons. Consistently, our in vivo study showed that Orai1 deficiency diminished PGE2-induced thermal hyperalgesia and mechanical allodynia. Overall, our findings demonstrate that peripheral Orai1 plays an important role in inflammatory pain and provide new insight into molecular mechanisms underlying peripheral sensitization.

Disclosures: Y. Mei: None. **D. Wei:** None. **H. Hu:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.05/I36

Topic: D.03. Somatosensation – Pain

Support: ENL20/18
Fondecyt 1191552

Title: The Cdk5/p35 complex increases P2X2/3R signaling in nociceptive trigeminal neurons

Authors: *E. UTRERAS[1], R. SANDOVAL[1], N. PINTO[1], P. CASTRO[2], C. GONZALEZ-BILLAULT[1], A. B. KULKARNI[3], R. MADRID[4], C. CODDOU[2];
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Abstract: We earlier reported that inflammation increases Cdk5 kinase activity, an important kinase involved in pain signaling. Cdk5 regulates channels expressed in nociceptive neurons, such as TRPV1, TRPA1 and the purinergic receptor P2X2, a ligand-gated ion channel involved in pain signaling, usually in the heterotrimeric form P2X2/3R. We also identified that Cdk5 phosphorylates an isoform of P2X2R, named P2X2aR in Thr372 causing a delay in use-dependent desensitization. Here in we report evaluation of the role of Cdk5 in the regulation of physiological function of P2X2/3R during orofacial pain. We performed Ca^{2+} imaging in primary culture of rat trigeminal ganglia (TG) neurons to determine P2X2/3R response to specific agonist named α,β-meATP. Then, we categorized calcium responses according to their kinetics and evaluated the effect of roscovitine, a pharmacological inhibitor of Cdk5. By voltage clamp, we analyzed the effect of Cdk5 activation on desensitization of P2X2aR mutants in T372. Finally, intradermal injection of α,β-meATP on whiskers pad were performed to evaluate facial pain-like behaviors in Cdk5 conditional KO mice (Cdk5 cKO). According to Ca^{2+} kinetics decay, two types of responses to α,β-meATP were found in TG neurons, a fast decay probably correspond to P2X3R homotrimer and a slow decay probably correspond to P2X2/3R heterotrimer. Interestingly, roscovitine treatment accelerates decay only from P2X2/3R activation. Moreover, disruption of putative Cdk5 phosphorylation site in P2X2aR was enough to lose Cdk5-dependent regulation of this channel. In addition, Cdk5 activation does not change P2X2aR surface distribution. On the other hand, we demonstrate that pain-like behaviors, as facial grooming and flinching, evoked by α,β-meATP injection in whiskers pad were significantly lower in Cdk5 cKO mice respect to littermates control mice. Altogether, our results demonstrate that Cdk5 inhibition was associated with reduced P2X2/3R activity, suggesting a Cdk5-mediated functional regulation of heterotrimer P2X2/3R in TG neurons. These results indicate that the P2X2R is a novel target of Cdk5. Moreover, Cdk5 cKO mice were less susceptible to nociceptive signaling dependent on P2X2/3R and P2X3R activation, suggesting an important role for Cdk5 in this pain signaling process.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.06/I37

Topic: D.03. Somatosensation – Pain

Support: BBSRC Research Grant BB/R006210/1
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Gates Cambridge

Title: Sephin1, a GMQ-related molecule, activates acid-sensing ion channel 3 (ASIC3) at neutral pH and enhances acid-mediated ASIC3 activation

Authors: *G. CALLEJO, J. R. HOCKLEY, L. A. PATTISON, J. C. GREENHALGH, S. CHAKRABARTI, E. S. SMITH, T. RAHMAN;
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Abstract: Acid-sensing ion channels (ASICs) are voltage-independent cation channels that detect decreases in extracellular pH. ASICs are involved in pain, fear, learning, neurodegeneration after ischemic stroke, and mechanosensation in the lower gastrointestinal tract. Of all the ASIC subunits, ASIC3 has been suggested as the key sensor of acid-induced pain and has also been shown to have a crucial role in different models of inflammatory pain including models of rheumatoid arthritis. Interestingly, ASIC3 has been proposed to play a dual role in arthritis: lack of ASIC3 ameliorates pain, but increases inflammatory processes in the arthritic joint. Therefore, the identification of new ASIC3 modulators and the mechanistic understanding of how these compounds modulate ASIC3 could be important for the development of new strategies to counteract the detrimental effects of dysregulated ASIC3 activity in inflammation. Here, we report the identification of novel ASIC3 modulators based on the ASIC3 specific agonist, 2-guanidine-4-methylquinazoline (GMQ). Through an in silico GMQ-guided screening of FDA-approved drugs, 5 different compounds were selected and tested for their modulation of ASIC3 using whole-cell patch-clamp. Of the chosen drugs, guanabenz, an α2-adrenoceptor selective agonist, produced similar effects to GMQ on ASIC3, activating the channel at neutral pH and potentiating its response to mild acidic stimuli. Sephin1, a guanabenz derivative that lacks α2-adrenoceptor activity, has been proposed to act as a selective inhibitor of a regulatory subunit of the stress-induced protein phosphatase 1 (PPP1R15A). However, we found that like guanabenz, sephin1 activates ASIC3 at neutral pH and potentiates its response to acidic stimulation, i.e. sephin1 is a novel modulator of ASIC3. Furthermore, docking experiments showed that guanabenz and sephin1 likely interact with the nonproton ligand-sensing domain of ASIC3. Overall, these data demonstrate the utility of computational analysis

for identifying novel ASIC3 modulators, which can be validated with electrophysiological analysis and may lead to the development of better compounds for targeting ASIC3 in the treatment of inflammatory conditions.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

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Topic: D.03. Somatosensation – Pain

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Title: Muscle GDNF signaling to neurons modulates peripheral sensitization after ischemic injury through a CREB/CBP interaction

Authors: *L. F. QUEME, A. A. WEYLER, M. P. JANKOWSKI;
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Abstract: Group III/IV primary muscle afferents transduce nociceptive stimuli in the periphery to the central nervous system, and also function as the sensory arm of the exercise pressor reflex (EPR), an increase in blood pressure and heart rate observed as a consequence of muscle activity. Clinical conditions associated with peripheral perfusion anomalies, such as peripheral artery disease or sickle cell anemia frequently present both muscle pain and alterations in the EPR, suggesting a role for primary sensory neurons in the development of these alterations post injury. Previously, we showed that mice exposed to peripheral ischemia with reperfusion injuries (I/R) have enhanced EPRs along with increased pain-related behaviors. These changes correlate with increased glial cell line derived growth factor (GDNF) in muscle tissue and upregulation of various genes in the affected dorsal root ganglia (DRGs) such as the GDNF family receptor α1 (GFRα1). Selective knockdown of GFRα1 in muscle afferents prevented I/R-induced upregulation of the purinergic receptor P2X5 and the acid sensing ion channel (ASIC) 3 in the DRG. Both of these channels are frequently associated with increased nocifensive behaviors and EPRs after ischemia. If and how GFRα1 modulates the expression of P2X5 and ASIC3, remains unclear. A transcription factor downstream of GDNF/GFRα1 signaling whose activity has been associated with P2X5 expression is cAMP response element-binding protein (CREB). We hypothesized that enhanced GDNF/GFRα1 signaling dually regulates I/R-induced pain-related behaviors and altered EPRs through CREB dependent transcription. To test this, we selectively knocked down GFRα1 via injection of siRNAs into the median and ulnar nerves *in vivo* prior to

unilateral forelimb I/R injury in mice. After 1d we performed pain-related behavioral tests, non-invasive cardiovascular measurements before and after exercise, and western blot (WB) analysis on the affected DRGs. I/R increased the expression of phosphorylated CREB (pCREB) and its coactivator CREB binding protein (CBP) in the DRGs. GFRα1 knockdown did not prevent the upregulation of pCREB but prevented the increased expression of CBP. Treatment of the I/R-affected mice with a CBP/CREB binding blocker at the time of injury prevented the development of pain-related behaviors, altered EPRs and increased P2X5 expression in the DRG. These results suggest GDNF signaling dually modulates pain-related behaviors and altered EPRs after I/R via group III/IV afferent sensitization through CREB/CBP activation. Data points to potential therapeutic targets for patients with ischemic myalgia.

Disclosures: L.F. Queme: None. **A.A. Weyler:** None. **M.P. Jankowski:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.08/I39

Topic: D.03. Somatosensation – Pain

Support: NIH MBRS-RISE: R25-GM059298
Genentech Foundation MS Dissertation Scholarship

Title: Feeding inhibits nociceptive responses during sensitization in the hornworm, Manduca sexta

Authors: *N. CRAWFORD[1], C. VALTIERRA[2], G. M. DOWNING[1], M. FUSE[1];
[1]Biol., San Francisco State Univ., San Francisco, CA; [2]Biol., San Francisco State, San Francisco, CA

Abstract: Sensory receptors are classified based on the receptor they respond to. Nociceptors are a class of sensory receptors that respond to damage to body tissue and are a component of pain perception in humans. Constant or extreme stimulation of these nociceptors with adequate stimuli would cause them to become hypersensitive, a term called nociceptive sensitization. Other physiological processes such as hunger can be distractions from this sensitization. In our model organism, the hornworm, Manduca sexta, I assessed the role of hunger and feeding on responsiveness during sensitization. M. sexta strikes defensively at a noxious stimulus, and using calibrated filaments applied to segments of the body wall, we have an in vivo method to determine the strike threshold as a gauge of a “pain-like” state. These filaments exert a set amount of force, providing an objective value to nociception. We assessed the threshold values in starved and fed larvae, and when food was introduced. We found that the threshold to strike was significantly increased when starved larvae were presented with food, but that the threshold

returned back to the hypersensitive state when the food was taken away. With the suggestion that feeding was the distracting physiological state, Octopamine, a neurohormone, and amine known for its role in altering food seeking behaviors were introduced into this experimental paradigm. The pharmacological effects of octopaminergic compounds will be discussed, to determine how over or under-expression of this neurohormone affects the threshold to strike. This data allows for a better understanding of how distracting stimuli affect nociceptors, allowing for better tool development for assessing pain perception in humans.

Disclosures: **N. Crawford:** None. **C. Valtierra:** None. **G.M. Downing:** None. **M. Fuse:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

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Program #/Poster #: 055.09/I40

Topic: D.03. Somatosensation – Pain

Support: Rita Allen Foundation/ American Pain Society
NINDS (R56NS103179-01)

Title: A novel interaction between the peripheral sensory, immune and endocrine systems modulates injury-related nociception in neonates

Authors: ***A. J. DOURSON**[1], C. E. MCCROSSAN[1], Z. K. FORD[1], M. C. HOFMANN[1], M. P. JANKOWSKI[1,2];
[1]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Dept. of Pediatrics, Univ. of Cincinnati, Cincinnati, OH

Abstract: Canonical growth hormone (GH) signaling involves the regulation of growth and homeostasis. However, recent clinical and preclinical evidence implicates an unexplored role for GH in nociception. Children deficient in GH may report peripheral pain, and our analysis of nociception in GH deficient animals found that these mice were hypersensitive to evoked stimuli specifically during early postnatal development (postnatal days 7 (P7)-P14) compared to controls. We also recently found that systemic pretreatment with GH could prevent inflammatory hyper-responsiveness. In addition, a local intramuscular injection of GH inhibited incision-related hypersensitivity in neonates during behavior and at the level of the primary afferent. This appeared to take effect through restoration of an injury-evoked decrease in tissue GH levels. The mechanism of GH-related anti-nociception, however, had not yet been determined. Here, we hypothesized that peripheral growth hormone signaling provides a tonic inhibition of afferent hypersensitivity that is transiently removed during postnatal injury. To test this hypothesis, we first knocked-out the growth hormone receptor (GHr) specifically in postnatal sensory neurons (Advillin-CreERT2; GHr flox mice) and performed behavioral analyses. Successful deletion of

GHr in primary sensory neurons resulted in hypersensitivity to peripheral stimuli in uninjured animals compared to controls. To further investigate the afferent mechanisms of GH action, we performed gene expression analysis of injured dorsal root ganglia (DRGs) and found GH treatment modulated the transcription factor serum response factor (SRF) after incision. Nerve-targeted, siRNA-mediated knockdown of SRF partially blocked incision-related hypersensitivity. The injury-induced decrease in GHr signaling to neurons was further found to regulate inhibitory microRNA expression, which may underlie the observed effects on DRG transcription. Finally, the incision-induced reduction in tissue GH levels was found to be due to the sequestering of this hormone by infiltrating macrophages. Targeted knock-out of the GHr specifically in macrophages, prevented incision-induced GH reductions and inhibited injury-related hypersensitivity and gene expression changes in the DRGs. Altogether, these results suggest that a unique immune cell-dependent displacement of GH in injured tissues sensitizes peripheral nociceptors leading to pain-related behaviors in neonates. Data may provide a novel strategy to treat pediatric pain.

Disclosures: **A.J. Dourson:** None. **C.E. McCrossan:** None. **Z.K. Ford:** None. **M.C. Hofmann:** None. **M.P. Jankowski:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.10/I41

Topic: D.03. Somatosensation – Pain

Support: 300008

Title: Up regulation of T type CaV3.2 channels by Cdk5 dependent phosphorylation shapes the compound action potential in afferent fibers and contributes to nerve injury induced allodynia

Authors: *K. GOMEZ[1], A. CALDERON-RIVERA[3], A. SANDOVAL[3], R. GONZALEZ-RAMIREZ[4], A. VARGAS-PARADA[1], R. DELGADO-LEZAMA[1], R. FELIX[2]; [1]Physiology, Biophysics and Neurosci., [2]Cell Biol., Cinvestav, Mexico City, Mexico; [3]Sch. of Med. FES Iztacala, Natl. Autonomous Univ. of Mexico (UNAM), Mexico City, Mexico; [4]Mol. Biol. and Histocompatibility, "Dr. Manuel Gea González" Gen. Hosp., Mexico CIty, Mexico

Abstract: Voltage-gated T-type calcium channels regulate diverse physiological events including neuronal excitability and have been linked to several pathological conditions such as absence epilepsy, cardiovascular diseases, and neuropathic pain. It is also acknowledged that calcium/Calmodulin-dependent protein kinase II (CaMKII) and protein kinases A and C (PKA and PKC) regulate the activity of T-type channels. Interestingly, peripheral nerve injury induces tactile allodynia and up-regulates CaV3.2 channels and Cdk5 in dorsal root ganglia (DRG) and

spinal dorsal horn (SDH). Here, we report that recombinant CaV3.2 channels expressed in HEK-293 cells are regulatory targets of cyclin-dependent kinase 5 (Cdk5). Site-directed mutagenesis showed that the relevant sites for this regulation are residues S561 and S1987. We also found that Cdk5 may regulate CaV3.2 channel functional expression in rats with mechanical allodynia induced by spinal nerve ligation (SNL). Consequently, the Cdk5 inhibitor olomoucine affected the compound action potential (cAP) recorded in the spinal nerves, as well as the paw withdrawal threshold. Likewise, Cdk5 expression was upregulated after SNL in the DRGs. These findings unveil a novel mechanism for how phosphorylation may regulate CaV3.2 channels and suggest that increased channel activity by Cdk5-mediated phosphorylation after SNL contributes nerve injury-induced tactile allodynia.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.11/I42

Topic: D.03. Somatosensation – Pain

Title: Fadu human squamous cell carcinoma induces hyperexcitability of primary sensory neurons

Authors: *M. L. UHELSKI[1], A. GORUR[2], Y. LI[3], P. M. DOUGHERTY[4], J. CATA[2]; [1]MD Anderson, Houston, TX; [2]MD Anderson Cancer Ctr., Houston, TX; [3]The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [4]Pain Med., Howard Hughes Med. Inst. - Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Cancer-related pain is a major concern for patients diagnosed with head and neck cancer. Symptoms can appear in the early stages of this disease, and more than 80% of patients report moderate to severe pain that can greatly diminish quality of life. Not only is this pain difficult to treat with currently available therapies, but these patients are also at a higher risk for post-treatment chronic pain. Tumors affecting this region can be highly inflammatory and impact sensory neurons innervating the area, even in the absence of perineural invasion. The mechanisms behind the development and maintenance of pain in early stage head and neck cancers are not fully understood. In the current study, we co-cultured human squamous cell carcinoma (FaDu, ATCC HTB-42, 100,000 per well) with rat dorsal root ganglion (DRG) neurons and performed whole-cell patch recordings after a 24 h incubation period in which the two cell populations occupied the same well but were not in direct physical contact. Neurons co-cultured with cancer cells had decreased rheobase compared to neurons cultured with media alone (177.8±44.7 pA vs 327.5±63.8 pA), and 26% demonstrated abnormal oscillations in

resting membrane potential while 21% had spontaneous discharge. Incubation with cancer-conditioned media (1 h) also induced changes in neuronal hyper-excitability, but no spontaneous activity was observed. Chemiluminescence assays indicated a high level of IL-6 release from FaDu cancer cells, and ELISA assays showed high levels of IL-6 in cancer-conditioned media. Compared to media taken from DRG only cultures, media taken from DRG and FaDu co-culture had elevated levels of a number of pro-inflammatory cytokines, including IL-6, as well as growth factors that have previously been shown to sensitize nociceptors. Elevated expression of IL-6 has been identified in the spinal cord and DRG in several experimental pain models, and administration of IL-6 is sufficient to induce hypersensitivity to mechanical and thermal stimuli. Pro-inflammatory factors released by the cancer cells could directly contribute to neuronal hyper-excitability, but may also indirectly contribute by inducing the release of additional pro-inflammatory factors from the neurons themselves and surrounding satellite cells. Our results demonstrate that Fadu cancer cells promote inflammation and nociception, and these effects can occur even before the tumor breaches nervous tissue. These results could help identify targets for therapies aimed at reducing pain in early stage head and neck cancer and may also be useful in cases where perineural invasion has occurred.

Disclosures: **M.L. Uhelski:** None. **A. Gorur:** None. **Y. Li:** None. **P.M. Dougherty:** None. **J. Cata:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: Arthritis Research UK RG20930
Rosetrees Postdoctoral Grant
Gates Cambridge Trust scholarship
BBSRC Doctoral Training programme
Arthritis Research UK RG21973
Department of Pharmacology University of Cambridge
Corpus Christi College Cambridge

Title: Modulation of knee-innervating dorsal root ganglion neurons with fibroblast-like synoviocytes and adeno-associated virus

Authors: *S. CHAKRABARTI[1], L. A. PATTISON[1], K. SINGHAL[1], R. H. RICKMAN[1], B. DOLESCHALL[2], J. R. F. HOCKLEY[1], G. CALLEJO[1], P. A. HEPPENSTALL[2], E. S. J. SMITH[1];

[1]Univ. of Cambridge, Cambridge, United Kingdom; [2]EMBL - Europaeisches Lab. Heidelberg, Monterotondo, Italy

Abstract: Arthritic pain is a major cause of disability that drastically reduces the quality of human life. We show that mice with complete Freund's adjuvant induced knee inflammation show a decrease in their natural digging behavior which is associated with an increase in excitability of knee-innervating dorsal root ganglion neurons. The chili pepper receptor, transient receptor potential vanilloid 1 (TRPV1), is also upregulated in these neurons and systemic administration of a TRPV1 blocker reversed inflammation induced decrease in digging behavior in mice. Mediators released by non-neuronal cells, such as fibroblast-like synoviocytes (FLS), in the joint can activate and/or sensitize sensory afferents and thus cause pain. We show here that primary FLS, derived from mouse knees, incubated in tumor necrosis factor-α (TNF-α, a key mediator in rheumatoid arthritis), results in the release of multiple inflammatory mediators, including interleukins 1β and 6. When knee neurons were co-cultured with these "inflamed" FLS, an increase in their excitability and TRPV1 activity was observed; co-culture with FLS not stimulated with TNF-α did not change neuronal excitability, i.e. inflammatory mediators released from "inflamed" FLS can directly sensitize knee neurons. These data suggest that knee neuron excitability influences arthritic pain and that specifically targeting knee-neurons could provide pain relief. Adeno-associated virus (AAV) mediated modulation of neuronal excitability is routinely achieved in the central nervous system, but efficient AAV delivery from the periphery to the DRG is challenging. Here we show that AAV-PHP.S robustly transduces ~5% lumbar DRG neurons from the knee and shows ~40% colocalization with the widely used retrograde tracer, fast blue. Our result suggests that AAV-PHP.S is a promising viral serotype for gene therapy in arthritic pain targeted to modulate sensory neurons from the knee.

Disclosures: S. Chakrabarti: None. **L.A. Pattison:** None. **K. Singhal:** None. **R.H. Rickman:** None. **B. Doleschall:** None. **J.R.F. Hockley:** None. **G. Callejo:** None. **P.A. Heppenstall:** None. **E.S.J. Smith:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.13/I44

Topic: D.03. Somatosensation – Pain

Support: Versus Arthritis RG20930
Versus Arthritis RG21973
Gates Cambridge Trust

Title: Human osteoarthritic synovial fluid increases excitability of mouse sensory neurones: An *in vitro* translational model to study arthritic pain

Authors: S. CHAKRABARTI[1], D. R. JADON[2], D. C. BULMER[1], ***E. S. SMITH**[1];
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Abstract: Knee osteoarthritis is a leading global cause of morbidity. Here we investigated the effects of knee synovial fluid obtained from patients with painful osteoarthritis (OA-SF) on the activity of knee-innervating sensory neurons as a novel translational model of disease mediated nociception in human osteoarthritis. Dissociated cultures of mouse knee neurons were incubated overnight or acutely stimulated with OA-SF and fluid from healthy donors (Ctrl-SF). Incubation with OA-SF induced knee neuron hyperexcitability compared to Ctrl-SF, such that the resting membrane potential significantly increased, and the action potential threshold decreased. Moreover, the number of neurons responding to agonists of TRPV1 and TRPM8 also increased. In response to acute application of Ctrl-SF and OA-SF, an increase in the intracellular Ca^{2+} concentration was observed. Responses to both Ctrl-SF and OA-SF were partially mediated via G_q intracellular signalling, whereas only responses to OA-SF were mediated via voltage-gated ion channels. In summary, OA-SF acutely activated knee-innervating neurons and induced hyperexcitability indicating that mediators present in OA-SF stimulate sensory neuron activity and thereby produce pain. Taken together, this study provides proof-of-concept for a new method to study the ability of mediators present in joints of patients with arthritis to stimulate nociceptor activity and hence identify clinically relevant drug targets for treating knee pain.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.14/DP04/J1

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.03. Somatosensation – Pain

Support: NIH, NCATS, R42 TR00127.

Title: Development of an *in vitro* human-derived 3D model of dorsal horn pain signalling

Authors: *W. A. ANDERSON[1,2], N. IYER[5], M. E. MESELHE[2], L. MCCOY[1], K. POLLARD[2], A. D. SHARMA[1], D. A. BOWSER[2,3], L. J. CURLEY[1], R. S. ASHTON[5], M. J. MOORE[1,2,4];
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Abstract: The “Opioid Crisis” of today emerged from the dramatic increase in prescription and misuse of highly addictive opioid compounds. Current models to study this area primarily depend on behavioral animal studies, which are expensive, time-consuming and do not always translate well in human clinical studies. This creates a need for physiologically relevant model systems for drug discovery and pre-clinical screening. A promising in vitro model involves the co-culture of rat sensory dorsal root ganglion (DRG) neurons with dorsal horn (DH) neurons of the rat spinal cord in a 2D culture system, where DRG neurons are found to synapse onto DH neurons, which simulates a major target for pain and opioid research. We are expanding upon this previous work by (1) developing this model in a 3D in vitro tissue format and (2) using human stem cell-derived neuronal cells to better recapitulate the in vivo environment and thus produce more reliable and translatable results for the future of pain research. The DHs and DRGs are isolated from embryonic rat spinal cords and cultured separately, as spheroids. These spheroids are then placed into micropatterned hydrogel constructs to induce organized and directed neuronal growth in 3D. Axons from the DRG grow through the 3D gel matrix, and we show evidence of synapse formation onto DH neurons, mimicking the native synapse structure found in the dorsal horn of the spinal cord in vivo. We characterize synapse formation through immunostaining the constructs for synapses and analyzing the synapse site through Synapsin I, CGRP, and MAP2 colocalization. Furthermore, we provide functional evidence of synapse formation through electrophysiological recordings of compound action potentials. This response was diminished when adding the synapse blocker CNQX, further suggesting synaptic communication. This initial 3D work with rat tissue laid the groundwork for incorporating human-derived tissue. We have created spheroids from both human sensory and DH neuronal progenitors and have successfully grown both spheroids in coculture in our dual hydrogel devices. This human 3D in vitro model of pain, structurally and functionally, approximates the in vivo DRG to DH sensory synaptic circuit - a critical gateway in transmission of pain stimuli. Our research aims to build a relevant platform for reliable and more rapid pre-clinical drug screening toward identification of the next generation of analgesics.

Disclosures: W.A. Anderson: None. **N. Iyer:** None. **M.E. Meselhe:** None. **L. McCoy:** None. **K. Pollard:** None. **A.D. Sharma:** None. **D.A. Bowser:** None. **L.J. Curley:** None. **R.S. Ashton:** None. **M.J. Moore:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.15/J2

Topic: D.03. Somatosensation – Pain

Title: Differential sensitization of lamina I and III-V spinoparabrachial (SPB) neurons in anesthetized mice in chronic inflammatory condition

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E-Phys, Clermont-Ferrand, France

Abstract: Sensitization of spinal lamina I projection neurons is claimed to be essential for the generation of chronic neuropathic and inflammatory pain. This claim was here assessed using extracellular recording of spinal lamina I and III-V projection neurons in vivo in an experimental model of chronic inflammation. Experiments were performed in ventilated, isoflurane-anesthetized, male Swiss mice, 24-48 h after injection of saline or CFA in one paw. Antidromic stimulations from the contralateral parabrachial area were used to search for SPB neurons. After recording of initial intrinsic activity, responses were evoked using camel brush, Von Frey hair (VF, 25, 50, 100, 200 mN), pinch with haemostat clamp, and water jet (WJ, 0, 24, 42, 46 and 50 °C). Blood pressure, body temperature and blood biochemistry were controlled to ensure adequate physiological status. A total of 152 SPB units were characterized. In control mice, lamina I and III-V neurons were essentially polymodal, with a few lamina I neurons being thermoreceptive, and some enigmatic lamina III-V neurons responding exclusively to noxious heat with delay. Polymodal neurons responding only to noxious stimuli were more abundant in lamina I than lamina III-V, whereas those responding to brush were found exclusively in lamina III-V. Pretreatment with CFA led to an overrepresentation of lamina I neurons responding preferentially to cold, and to an increase of the intrinsic activity and occurrence of responses to brush of lamina I and III-V neurons. “Heat delayed” lamina III-V neurons were not found in CFA pretreated mice. For lamina I neurons, statistical analysis demonstrated a significant increase of the number of action potential and peak firing frequency of the intrinsic activity and evoked responses to brush, VF25-200 mN, and WJ at 0 and 24 °C in CFA pretreated compared to control mice. In comparison, for lamina III-V neurons, the number of action potential and peak firing frequency were significantly increased for brush, VF 200 mN and WJ 24 °C. Central conduction velocities, which were significantly faster in lamina III-V compared to lamina I neurons, were similar in CFA pretreated and control mice. The present data confirmed a primary role of lamina I SPB neurons in the generation of cold and mechanical allodynia/hyperalgesia in chronic inflammation. An additional participation of lamina III-V SPB neurons in the generation of mechanical allodynia was also suggested. The technique described herein, allowing the recording of unambiguously identified SPB neurons, could take advantage of genetically modified mice or be used to assess the efficacy of analgesic drug candidates.

Disclosures: J. Allard: None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

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Program #/Poster #: 055.16/J3

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01DA37621
NIH Grant R01NS45954

Title: A-type potassium currents mediated by Kv4.2 regulate excitability of Y1R-eGFP expressing dorsal horn neurons after nerve injury

Authors: *G. P. SINHA, B. K. TAYLOR;
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Abstract: Nerve injury induces pain-related behaviors thought to be driven by sensitization of dorsal horn neurons. Injury-induced changes in the membrane properties of these neurons and their synaptic networks are not well-understood, perhaps due to their complex heterogeneity. We focused our attention on an interneuronal subpopulation that express the neuropeptide Y (NPY) Y1-receptor. We recently reported that these neurons co-express markers of excitatory but not inhibitory interneurons, and that Y1R-selective neurotoxin ablation reduced behavioral signs of neuropathic pain (Nelson et al, Sci Reports, 2019). Last year at SFN 2018, we reported that 74% of Y1R neurons exhibit delayed firing upon current injection. The Y1R-eGFP neurons exhibited two types of A-type currents that underlie delayed firing - 50% with fast (~30ms) and 24% with slow (>100 ms) decay constants. 56% of Y1-eGFP expressing neurons co-localized with Kv4.2 antibodies. We also presented initial results on subthreshold A-type currents after injury. Based on these results, we hypothesized that nerve injury down-regulates Kv4.2, leading to shift in active membrane properties of Y1R neurons towards increased excitability. To test this, we recorded isolated A-type currents during steady state activation / inactivation and recovery from inactivation in Y1R-eGFP neurons in the presence of TTX (2 µM), TEA (10 mM) and Cd^{2+} (200 µM), further isolated by a two-step voltage protocol (Hu et al. 2006). In additions to different activation/inactivation thresholds, the slopes of the activation and inactivation curves were also different between fast and slow A-type currents, suggesting that the fast and slow A-type currents are distinct. 4-aminopyridine (4-AP), a K-channel blocker, inhibited both fast and slow A-type isolated currents, although at different concentrations. Compared to naive mice, SNI mice exhibited several significant changes in a subpopulation of neurons exhibiting fast A-type currents - (1) a depolarized shift in resting membrane potential; (2) a hyperpolarized shift in AP threshold; and (3) more importantly increase in rebound spiking. Nerve injury produced a hyperpolarizing shift in the inactivation curve of A-type currents. Recovery of A-type currents from inactivation was also quicker. We speculate that peripheral nerve injury reduces the participation of available Kv4.2 channels, therefore reduces the effect of A-type current and cause a shorter delay in AP firing from onset of current injection. These along with changes in membrane properties suggest that SNI increase excitatory output from the glutamatergicY1R-eGFP neurons, leading to neuropathic hypersensitivity.

Disclosures: G.P. Sinha: None. **B.K. Taylor:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.17/J4

Topic: D.03. Somatosensation – Pain

Support: NS091296 (FW)

Title: Compositional changes in the gut microbiome contribute to stress-induced comorbid visceral pain

Authors: *J. ASGAR[1], J. YANG[1], J. RAVEL[2], R. TRAUB[1], W. GUO[1], S. ZOU[1], R. DUBNER[1], K. REN[1], F. WEI[1];
[1]Univ. of Maryland Sch. of Dent., Baltimore, MD; [2]Inst. for Genome Sci., Baltimore, MD

Abstract: Chronic overlapping pain conditions (COPCs) including temporomandibular disorder, irritable bowel syndrome (IBS) and chronic low back pain are highly prevalent in women and are commonly associated with psychological stress. We have previously reported that in rats with existing orofacial neuropathic pain, 3-day repeated forced swimming stress leads to visceral hypersensitivity. Referred hyperalgesia in the low back area is more persistent in female rats than males. It is associated with a long-lasting upregulation of 5-HT3A receptors in the lumbosacral spinal cord and dorsal root ganglia. Intrathecally administered 5-HT3R antagonist transiently blocks referred hyperalgesia, suggesting 5-HT3R-mediated sensitization of spinal primary afferents from the gut. However, the underlying mechanisms are still unclear. This study expands on our previous observation that repeated forced swimming leads to gut dysbiosis in rats with orofacial pain. Using 16S rRNA-based analysis, we confirmed extensive compositional changes in fecal microbiota of rats with comorbid pain after stress. We found that some changes persisted for weeks, including an increase in the relative abundance of Firmicutes and a decrease in Bacteroidetes. Other changes were time dependent: for example, Proteobacteria were increased 5d after stress but reduced at 14d and 21d compared with baseline. We also found a reduction in microbiome biodiversity. Finally, fecal microbiota transfer (FMT) from rats with referred hyperalgesia induced comorbid pain in recipient rats with orofacial pain alone at 1-3 weeks after FMT. However, naïve recipients did not develop this phenotype. These data suggest that ongoing orofacial pain is a prerequisite for developing comorbid pain. Conversely, FMT from naïve rats attenuated referred hyperalgesia in recipient rats with comorbid pain 1-3 weeks after FMT. These findings indicate that compositional changes in the gut microbiome contribute to stress-induced persistent referred hyperalgesia in rats with orofacial pain. In sum, this study provides compelling evidence for time-dependent compositional changes in the gut microbiota of female rats with comorbid visceral pain. These changes are distinct from those associated with orofacial pain or stress alone. Based on our FMT studies, the observed dysbiosis and comorbid visceral

pain are causally related. Further understanding of this causal relationship will help advance novel therapeutic strategies for comorbid pain conditions.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

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Program #/Poster #: 055.18/J5

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 DK103769

Title: Aoah mediates gut microbiome modulation of pelvic pain

Authors: *W. YANG[1], R. YAGGIE[1], J. ROSEN[1], C. BUSHELL[2], M. WELGE[2], B. WHITE[2], A. SCHAEFFER[1], D. J. KLUMPP[1];
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Abstract: Interstitial cystitis/bladder pain syndrome (IC) is a chronic condition associated with severe pelvic pain and voiding dysfunction, and recent studies indicate fecal dysbiosis in female patients with IC. Using a murine model of IC, we recently identified the locus encoding acyloxyacyl hydrolase, *Aoah*, as a modulator of pelvic pain severity where AOAH-deficient mice develop spontaneous pelvic pain and increased response in induced pelvic pain models. Here, we report that AOAH-deficient mice also exhibit dysbiosis of GI microbiota. AOAH-deficient mice exhibit an enlarged cecum, a phenotype long associated with germ-free rodents, and increased mass of cecal contents. 16S rDNA sequencing of cecal contents and stool revealed altered microbiota in AOAH-deficient mice, and LC-MC revealed altered metabolomics. Transepithelial resistance was significantly lower in the cecum of AOAH-deficient mice, suggesting a “leaky gut” phenotype, and transcriptome analyses indicated altered cytoskeletal remodeling pathways. Co-housing AOAH-deficient mice with wild type mice resulted in converged microbiota. Co-housing with wild type mice also abrogated the pelvic pain phenotype of AOAH-deficient mice, a finding that was corroborated by gavage of AOAH-deficient mice with stool slurry of wild type mice. Together, these data indicate that AOAH mediates normal gut microbiota, and the dysbiosis associated with AOAH deficiency causes pelvic pain. Gut flora may be a potential therapeutic target for IC.

Disclosures: W. Yang: None. **R. Yaggie:** None. **J. Rosen:** None. **C. bushell:** None. **M. Welge:** None. **B. White:** None. **A. Schaeffer:** None. **D.J. Klumpp:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.19/J6

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS045594
NIH Grant NS055860
NIH Grant AR068989

Title: Sensory neuron, sympathetic preganglionic and post ganglionic neurons form a loop that may amplify neuropathic pain in the spared nerve injury model

Authors: *W. XIE[1], J. A. STRONG[1], J.-M. ZHANG[2];
[1]Anesthesiol., Univ. Cincinnati Coll Med., Cincinnati, OH; [2]Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Sympathetic activity can potentiate neuropathic pain. In our previous work using the spinal nerve ligation model we found that sympathetic activity directly increased excitability of nerve-injured DRG neurons, increased local DRG inflammation, and reduced the pain-associated peripheral nerve regeneration process. In this study we used the spared nerve injury (SNI) neuropathic pain model. We observed that cutting the grey rami (postganglionic sympathetics) to the L4 and L5 spinal nerves reduced spontaneous, mechanical and cold pain behaviors. This effect was partly due to reduction of regeneration, as shown by reduced immunostaining for regeneration marker GAP43 in both neuroma and DRGs. We previously showed using in vivo recording that activation of sympathetic preganglionic axons could be increased by electrically stimulating the sciatic nerve in both normal and, to a greater degree, SNI rats. To determine if output from sympathetic preganglionic neurons played any role in the observed effects of cutting the grey rami at L4 and L5, we cut the 2 most caudal white rami (preganglionic axons) and their associated grey rami (at the level L3/L2 level), leaving the postganglionic sympathetic fibers to the L4 and L5 spinal nerves intact but with reduced preganglionic input. This procedure decreased mechanical hypersensitivity after SNI, and reduced size and GAP43 expression of the neuroma (28 and 56 days later), suggesting decreased nerve regeneration. On day 14, sympathetic sprouting in both DRG and neuroma was reduced. Finally, the frequency and amplitude of postganglionic sympathetic activity recorded in vivo from the grey ramus at L4 in SNI rats was reduced by white rami cutting. The results suggest that sympathetic activation strongly depended on output from preganglionic neurons, which in turn could be activated by noxious sensory input. Under neuropathic pain conditions, sympathetic activation not only contributed to hypersensitivity of injured sensory neurons but also was enhanced by the abnormal painful inputs. Inhibiting the loop formed by injured peripheral nerve, sympathetic

preganglionic neurons and sympathetic post ganglionic neurons may help to reduce neuropathic pain.

Disclosures: **W. Xie:** None. **J.A. Strong:** None. **J. Zhang:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.20/J7

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS045594
NIH Grant NS055860
NIH Grant AR068989
National Natural Science Foundation of China 81400916

Title: High fat diet exacerbates neuropathic pain behaviors by delaying regeneration in a crush nerve injury model

Authors: X. ZHU[1,2], W. XIE[1], J. A. STRONG[1], *J.-M. ZHANG[1];
[1]Anesthesiol., Univ. of Cincinnati Col. of Med., Cincinnati, OH; [2]Anesthesiology, Xiangya Hosp., Central South Univ., Changsha, China

Abstract: Our previous studies showed that high fat diet exacerbates inflammatory pain, but less is known about its effects on neuropathic pain. Previously, we showed that pain behaviors are linked to the peripheral nerve regeneration process in several neuropathic pain models. In the long-lasting spared nerve injury model (SNI), target reinnervation is not possible and the futile regeneration process results in neuroma formation. This model also gives maximal mechanical hypersensitivity, making it difficult to observe exacerbation by high fat diet. In this study, effects of diet were explored in modified model, in which the tibial nerve was crushed near the sciatic trifurcation, rather than being ligated. This resulted in submaximal mechanical hypersensitivity (von Frey test) that peaked at day 3 and recovered in 3 - 4 weeks. To study effects of diet, Sprague-Dawley rats (both sexes) were fed a high fat diet (HFD: 40% calories from butter fat) or normal chow (NC). Compared with NC rats, rats fed HFD starting 6 weeks before the tibial crush surgery had increased mechanical hyperalgesia and delayed recovery. The largest diet effect on von Frey sensitivity was observed on day 3. Immunostaining of the nerve regeneration marker GAP43 in both L4 DRG and nerve crush site was elevated on day 3 and 7 in NC rats, but this elevation was delayed until day 7 in the HFD rats (n=4 rats per group). The pan-macrophage marker Iba-1 immunostaining also increased after nerve crush in both the DRG and the nerve crush site on day 3, with larger increases in the HFD group (n=4 rats per group). qPCR showed that the expression of pro-inflammatory macrophage marker TNF-α is up-regulated in the DRG

in the HFD group prior to nerve crush, and increased further in both groups after nerve crush. Immunostaining of the type 1 pro-inflammatory cytokine CCL2 (MCP-1; a key macrophage chemoattractant) increased in both DRG and nerve crush site 3 days after nerve crush in both diet groups, but with larger increases in the HFD group (n=4 rats per group), suggesting CCL2 may mediate some effects of both diet and nerve crush on pain behaviors. Consistent with this, preliminary results suggested that knockdown of CCL2 (via siRNA injection into L4 and L5 DRG just prior to nerve crush) reduced pain behaviors induced by nerve crush. Nerve regeneration and resolution of pain behaviors involve a transition from type 1 to type 2 inflammation; we propose that HFD may increase type 1 inflammation at baseline and early after nerve crush, exacerbating pain and delaying the regeneration and recovery from pain in this transient neuropathic pain model.

Disclosures: X. Zhu: None. **W. Xie:** None. **J.A. Strong:** None. **J. Zhang:** None.

Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.21/J8

Topic: D.03. Somatosensation – Pain

Title: The role of alpha-synuclein in inflammatory and neuropathic nociception in mice

Authors: *M. MÖLLER[1], C. V. MÖSER[1], G. GEIßLINGER[1,2], E. NIEDERBERGER[1]; [1]pharmazentrum Frankfurt/ZAFES, Inst. für Klinische Pharmakologie, Klinikum der Goethe-Universität, Frankfurt am Main, Germany; [2]Project Group for Translational Med. & Pharmacol. TMP, Fraunhofer Inst. for Mol. Biol. and Applied Ecology IME, Frankfurt am Main, Germany

Abstract: Alpha synuclein (α-Syn, SNCA) is a neuronal protein mostly known for its role in Parkinson's disease. There it makes up large parts of Lewy bodies. Other functions include recycling of the neuronal transmitter pool and contribution to the release of dopamine. The results of our work hint to a so far unknown new function of α-Syn indicating that it also plays a role in the transmission of noxious stimuli. This assumption is supported by the localization of α-Syn in the spinal cord of mice. The results of our immunohistochemistry experiments show that it is localized in the dorsal horn laminae I and II. Here, afferent nociceptive nerve fibers form synapses with second order neurons which transmit noxious stimuli into the brain. In behavioral experiments, we assessed the nociceptive reaction in response to different stimuli in SNCA knockout ($SNCA^{-/-}$) animals compared to wildtype littermates (WT). The deletion of α-Syn had no impact on motoric function and reaction to mechanical stimulation. In models of acute nociception, $SNCA^{-/-}$ animals have a higher acceptance for noxious low temperatures but showed no differences in mechanical and heat nociception. The formalin-induced paw inflammation was used as a model of central sensitization. In this model, $SNCA^{-/-}$ mice revealed a reduced

nociceptive response in comparison to wildtype mice. In contrast, in the zymosan-induced paw inflammation model which lasts longer than the formalin model and shows cardinal symptoms of inflammation, we observed no differences between the genotypes. These results indicate that α-Syn is involved in central sensitization rather than the inflammatory response. The nociceptive response in the formalin model was associated with a transiently increased α-Syn protein level in the spinal cord of WT animals. Reduced nociception in $SNCA^{-/-}$ mice might be due to differential neurotransmitter release or reduced upregulation of specific inflammatory marker proteins. In summary, our results indicate that α-Syn participates in nociceptive pathways which might open up new avenues in pain research.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

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Program #/Poster #: 055.22/J9

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS045594
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NIH Grant AR068989

Title: Steroid receptor regulation of inflammatory low back pain in mice

Authors: *K. A. QUALLS[1], L. ZHANG[1,2], S. I. IBRAHIM[1], D. BUESING[1], Y. M. ULRICH-LAI[1], W. XIE[1], J. A. STRONG[1], J.-M. ZHANG[1];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Beijing Friendship Hosp., Capital Med. Univ., Beijing, China

Abstract: Low back pain (LBP) is a prevalent and costly condition, often caused by inflammation at the dorsal root ganglion (DRG). Epidural steroid injections (ESIs) are commonly used to relieve this inflammation, though they have varied clinical efficacy. While the desired target of ESIs at the DRG is the glucocorticoid receptor (GR), evidence suggests that many steroids used in ESIs have off-target effects at the mineralocorticoid receptor (MR). While the GR is known to relieve inflammation, the MR has been shown to be pro-nociceptive in the rat DRG. In the Local Inflammation of the DRG (LID) model of low back pain in rats, protein expression of the GR was shown to be downregulated, while the MR was upregulated. Both GR and MR bind endogenous corticosterone in rodents, which is converted to its active form by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), and deactivated by 11β-HSD2. 11β-HSD1 and 11β-HSD2 serve as important local regulators of GR and MR activity. Both enzymes are

present in rat DRG, where 11β-HSD1 mRNA was upregulated after LID. **In this study, we aim to characterize expression of GR and MR, as well as 11β-HSD1 and 11β-HSD2, in the mouse DRG in a model of inflammatory LBP.** The LID model was implemented in 8-12 week old C57BL/6J mice. Mice in the LID group received a 3 μL injection of zymosan (2 mg/mL) over the right L3 and L4 DRGs, while sham mice received the same procedure without injection. As in rats, this model induced long-lasting mechanical hypersensitivity in mice. Protein expression patterns and levels were assessed using immunohistochemistry and Western blotting. Sham mice served as controls, and males and females were analyzed separately, before combining data that did not show a sex-dependent effect. Immunohistochemical staining showed that GR and MR are expressed in normal mouse DRG neurons. However, on postoperative day 1 after LID, the GR was downregulated in the DRG in comparison to Sham mice. These results indicate that steroid receptor expression is dynamic during inflammatory LBP, which could explain, in part, the varied efficacy of ESIs. This work could support future functional analysis of steroid receptors and their local regulation in the DRG, and aid in understanding how to improve ESIs for the treatment of LBP.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

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Program #/Poster #: 055.23/J10

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS045594
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Title: Functional macrophage subtype changes in a mouse model of inflammatory low back pain/radiculopathy

Authors: L. ZHANG[1,2], W. XIE[1], S. H. LEE[1], T. BERTA[1], *J. A. STRONG[1], J.-M. ZHANG[1]; [1]Anesthesiol., Univ. of Cincinnati Col. of Med., Cincinnati, OH; [2]Beijing Friendship Hosp., Capital Med. Univ., Beijing, China

Abstract: Macrophages play key roles in many pain states. They have been broadly classified as M1 or pro-inflammatory, and M2 or anti-inflammatory/involved in tissue repair. Macrophages can change their polarization in response to the local tissue environment. Our previous studies in rat showed that macrophage density (pan-macrophage Iba1 labeling) increased in the DRG after localized inflammation of the DRG (LID; a low back pain/radiculopathy model), but little is

known about the functional macrophage subtypes involved in low back pain. In this study, we examined how different functional macrophage subtypes were involved in the LID model. The CX3CR1 $^{eGFP/+}$ CCR2 $^{RFP/+}$ transgenic mouse model was used to separately label CX3CR1-expressing (primarily resident) macrophages and CCR2-expressing (primarily infiltrating) macrophages. The LID model was established by a 3 μl immune activator zymosan (2 mg/ml) injection into the right L4 intervertebral foramen, over the DRG. Local DRG inflammation caused mechanical hypersensitivity and increased guarding (spontaneous pain) in the ipsilateral paw, as previously shown for rats. Quantitative microscopy revealed that infiltrating macrophages increased after LID in the inflamed DRG on day 4, 7 and 14. Liposomal clodronate or vehicle (200 μl) was injected intravenously 2 days before LID surgery in order to deplete macrophages. After clodronate, the mice had less LID-induced mechanical hypersensitivity and spontaneous pain, compared with i.v. vehicle-injected mice. We found it feasible to amplify specific macrophage polarization markers with qPCR using small samples of individually identified fluorescently labeled macrophages isolated from blood or DRG, which will enable further characterization of the polarization state of these two macrophage subtypes in this model over time. Overall, our results to date suggest that infiltrating macrophages may contribute to inflammatory pain in a mouse model of low back pain and radiculopathy.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.24/J11

Topic: D.03. Somatosensation – Pain

Title: The effect of orthodontic tooth movement on the excitability of the trigeminal ganglion

Authors: *M. YUGAWA[1], T. TSUCHIYA[1], N. HASEGAWA[1], A. SASAKI[1], N. SUDA[1], K. ADACHI[2];
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Abstract: During orthodontic treatment, pain is induced by tooth movement. It is well known that peripheral pain induces excitation of the satellite glial cells, which is associated with expression of GFAP (Glial fibrillary acidic protein), in the ganglion of the primary afferent fibers. Therefore, relationship between the expression of GFAP-positive satellite glial cells and orthodontic force induced pain was investigated, in this study. We used experimental tooth movement model which allowed us to evaluate orthodontic force-induced pain, by reduction of the threshold for inducing jaw-opening reflex (JOR-TH), and other biological (e.g.,

electrophysiological, histological and biochemical) features, simultaneously. Anesthetized male Wister rats were placed by an orthodontic brace and a Ni-Ti coil spring between bilateral incisors and the right maxillary first molar to apply the continuous orthodontic force. The analgesic administrated groups were received intraperitoneal injection (3 times/day, 8 hrs interval for one day) of aspirin (100 mg/kg) or morphine (1 mg/kg) immediately after orthodontic force application. One (D1), 3 (D3) or 7 (D7) days after orthodontic force application, the orthodontic force-induced pain was evaluated by measurement of JOR-TH under general anesthesia. After JOR-TH evaluation, animals were perfused with 4% paraformaldehyde, then bilateral trigeminal ganglia were removed and kept in same fixative solution for additional one day. Subsequently, sampled tissue was dehydrated by sucrose and horizontally sectioned (5 μm). Immunofluorescence staining for GFAP was performed for the sections, and observed. The number of somata of trigeminal nerve surrounded by GFAP-immunoreactive (IR) cells more than 2/3 of its perimeter in I, II and III branch regions of the trigeminal ganglia was measured. It has been established that the right JOR-TH was significantly reduced compared with that of left at D1 in this model, and the right TH was recovered at D3 and exceeded at D7. There were significant increase of GFAP-IR cells-surrounded somata in brunches I and II of right side compared with those of left side at D1, however, there were no significant differences at D3 or D7. Moreover, treatment of both aspirin and morphine significantly increased the right JOR-TH at D1 and that was associated with significant reduction of the expression of GFAP-IR cells-surrounded somata in brunches I and II of right side compared with those of contralateral side. This result indicated that orthodontic force-induced pain was accompanied with satellite glial activity in the trigeminal ganglia.

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Poster

055. Pain: Channels and Physiology Afferents to Spinal Cord

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 055.25/J12

Topic: D.03. Somatosensation – Pain

Support: Western Sydney University School of Medicine

Title: Minocycline reduces experimental muscle hyperalgesia induced by nerve growth factor in humans: A placebo-controlled double-blind drug-crossover study

Authors: *J. S. DUNN[1], S. S. NAGI[2], D. A. MAHNS[1];
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Abstract: Hyperalgesia is a heightened pain response to a stimulus which would normally be perceived as painful and is a hallmark of many common neuropathic and chronic pain conditions. In a double-blind placebo-controlled drug-crossover trial the effects of concomitant and delayed minocycline treatment on the initiation and resolution of muscular hyperalgesia were tested. In an untreated cohort, repeated injections (5μg: days 0, 2 and 4) of nerve growth factor (NGF) in the flexor carpi ulnaris muscle of the forearm produced a peak (at day 7) increase in muscular hyperalgesia which was measured as a fall in pressure pain thresholds across the anterior forearm and lasted for 2 weeks. Participants who received placebo in week 1 exhibited the same increase in muscle hyperalgesia at day 7 but significantly reduced muscular hyperalgesia following minocycline treatment in week 2 (day 7: 200mg then 100mg b.i.d. for 7 days). Participants who received minocycline during week 1 (day 0: 200mg then 100mg b.i.d. for 7 days) developed significantly less (~50%) hyperalgesia by day 7 compared to untreated and placebo counterparts. However, the switch to placebo in week 2 led to the same proportional degree of recovery from peak hyperalgesia as the untreated cohort. In this double-blind crossover study, concomitant (week 1) minocycline treatment reduced the regional extent and intensity of NGF-induced mechanical pain hyperalgesia. Delayed minocycline treatment (week 2) enhanced recovery of an established muscular hyperalgesia. The design of this study allows the observation that regardless of time of administration relative to pain hypersensitivity inducement, minocycline treatment is able to evoke an efficacious outcome.

Disclosures: **J.S. Dunn:** None. **S.S. Nagi:** None. **D.A. Mahns:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.01/J13

Topic: D.03. Somatosensation – Pain

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001
Sao Paulo Research Foundation (FAPESP) [grant number 2017/17919-8]
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Title: Physical exercise prevents transition of acute to chronic muscle pain by a mechanism dependent of PPARγ receptors

Authors: *M. G. OLIVEIRA-FUSARO[1], G. AZAMBUJA[1], C. O. JORGE[1], B. B. GOMES[1], H. L. RODRIGUES[1], C. FUSARO[2];
[1]Lab. of Pain and Inflammation Research, Sch. of Applied Science, State Univ. of Campinas, Limeira, Brazil; [2]Sao Francisco Univ., Bragança Paulista, Brazil

Abstract: Acute and chronic pain are widely studied, however the mechanisms underlying transition of acute to chronic pain are poorly understood. Physical exercise and activation of PPARγ receptors are able to control pain conditions. Interestingly, physical exercise increases the expression of PPARγ receptors in muscle tissue. Therefore, the aim of this study was to evaluate whether physical exercise and/or activation of PPARγ receptors prevents transition of acute to chronic muscle pain and, if so, whether physical exercise prevents this transition by a mechanism dependent of PPARγ receptors. Male Swiss mice (2 months old) were used and all procedures were approved by ethics committee for animals at UNICAMP (protocol 4808-1). Carrageenan (100µg) was injected into gastrocnemius muscle to induce inflammatory pain and, 10 days later, PGE_2 (1µg) was injected at the same local to reveal the state of chronic-latent hyperalgesia. GW9662 (9ng), a selective PPARγ receptor antagonist, and $15dPGJ_2$ (100ng), a non-selective PPARγ receptor agonist, were used. Mechanical muscle hyperalgesia was measured by Randall-Selitto analgesymeter in different time points of acute (1-144h) and chronic pain (1-168h). Physical exercise was performed through swimming, in a volume of 50 min/day, 5 days/week, for 3 weeks, before carrageenan injection. Once a week, blood was collected to assay corticosterone levels by ELISA test because exercise-induced analgesia can be modulated by stress. Tester was blinded to all groups, statistical analysis was performed by Two Way ANOVA and Bonferroni post test, and significance was set at $p<0.05$. Results showed that $15dPGJ_2$ prior to carrageenan reduced acute muscle hyperalgesia and prevented chronic muscle hyperalgesia (n=5). To isolate acute from chronic muscle hyperalgesia, $15dPGJ_2$ was injected immediately before PGE_2 in mice previoulsy challenged by carrageenan. In this case, $15dPGJ_2$ reduced the intensity of chronic muscle hyperalgesia (n=5). Swimming prevented acute and chronic muscle hyperalgesia (n=14), without affect levels of corticosterone (n=10), and GW9662, prior to carrageenan, reverted these preventions (n=5). These data demontrate that activation of PPARγ prior to an inflammatory insult prevents transition of acute to chronic muscle pain and, post the insult, reduces the intensity of chronic muscle pain. In addition, exercise prevents transition of acute to chronic muscle pain by a mechanism dependent of PPARγ. Our findings point out physical exercise as a good strategy to prevent transition of acute to chronic muscle pain, and suggest PPARγ receptors as a pharmacological target to potentiate the hypoalgesic effect of exercise.

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Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.02/J14

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS108087

Title: Peripheral knockdown of endocytic protein AP2A2 ameliorates acute and chronic inflammatory pain-like behaviors in mice

Authors: *R. G. POWELL[1], A. BHATTACHARJEE[2];
[1]SUNY Univ. At Buffalo, Buffalo, NY; [2]Univ. at Buffalo - The State Univ. of New York, Buffalo, NY

Abstract: Chronic pain is one of the most debilitating diseases in the United States, yet, current treatment strategies are not suited for long-term pain relief, necessitating the identification of additional molecular targets to develop novel pain-killing drugs. Nociceptive dorsal root ganglion (DRG) neurons remain central sites for investigative study as neuronal plasticity underlies pain states. During tissue damage, inflammatory mediators initiate signal transduction in DRG neurons—altering channel properties and concomitant pain perception. Chronic exposure to inflammatory mediators leads to thermal hyperalgesia and mechanical allodynia. We have previously documented the relationship between protein kinase A activation and adaptor protein complex 2 (AP2) clathrin-mediated endocytosis (CME) of the sodium-activated potassium channel, Slack, in cultured DRG neurons (Gururaj et al., JBC 2017). Here we demonstrate that peripherally interfering with the AP2 complex strikingly alters pain behavior. Utilizing a novel spinal nerve injection technique, shRNAs targeted against the alpha-2 subunit (AP2A2) of the AP2 complex was unilaterally transfected *in vivo* into the sciatic nerve of naïve C57BL/6 male and female mice and pain behaviors were assed during acute and chronic inflammatory pain. In a model of acute inflammatory pain, mice exhibited a significant reduction in nocifensive behaviors (spontaneous licking, and paw lifts) during AP2A2 deficiency. In a chronic inflammatory pain model, mice exhibited a robust increase in paw withdrawal latency during thermal behavioral testing when AP2A2 was preemptively knocked down, suggesting that AP2-CME is required for the initiation of chronic pain states. Furthermore, after chronic pain was established knockdown of the AP2A2 subunit rapidly reversed hyperalgesia, suggesting that AP2-CME is required for maintenance of chronic pain states. These data suggest that extra-synaptic AP2-CME is a critical process for the initiation and maintenance of peripheral nociceptor sensitization and inflammatory pain behavior.

Disclosures: R.G. Powell: None. **A. Bhattacharjee:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.03/J15

Topic: D.03. Somatosensation – Pain

Title: SUVN-K1806045: A P2X7 receptor antagonist, in animal models of nociception and peripheral neuropathic pain

Authors: V. GOURA, A. VUYYURU, R. KALLEPALLI, R. ABRAHAM, K. BOJJA, V. REBALLI, P. JAYARAJAN, S. KOMMINENI, S. JARUGUMALLI, *N. MUDDANA, R. NIROGI;
Suven Life Sci. Ltd., Hyderabad, India

Abstract: Chronic nociception and peripheral neuropathic pain are still in focus for better therapy in clinic, as current therapy is far from satisfactory. Patients suffering from chronic nociceptive and neuropathic pain expressed significant increase in levels of purinoreceptors P2X7 and interleukin 1 beta (IL-1β) release. P2X7 receptors (P2X7R) are involved in regulation of inflammation, diabetic neuropathy, and chronic nociception. Thus, inhibition of P2X7R may be an option to treat this kind of frequently intractable pain. P2X7R antagonists were designed and developed and among those SUVN-K1806045 was evaluated in animal models of formalin induced nociception (FIN) and streptozocin induced diabetic peripheral neuropathy (DPN) for its analgesic activity. Behavioral parameters like flinching and licking of paw were evaluated in FIN. Paw withdrawal thresholds were evaluated using Von Frey monofilaments in DPN. SUVN-K1806045 was evaluated for modulation of agonist induced IL-1β increase. SUVN-K1806045 showed significant reduction in number of flinches and duration of licking of paw in FIN. Statistically significant increase in paw withdrawal thresholds were observed in SUVN-K1806045 treated DPN rats when compared with vehicle treated group. These results suggest that, SUVN-K1806045 would be a promising candidate for treating chronic nociception and neuropathic pain.

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Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.04/J16

Topic: D.03. Somatosensation – Pain

Support: NIH DE016062

Title: Role of DNA methylation in transcriptional regulation of pro-nociceptive genes implicated in inflammatory muscle pain in trigeminal ganglia (TG)

Authors: *J. JOSEPH, G. BAI, H. ROSS, Y. ZHANG, M.-K. CHUNG, K. S. LEE, J. Y. RO; Neural and Pain Sci., Univ. of Maryland Baltimore, Sch. of Dent., Baltimore, MD

Abstract: Inflammation and injury produce rapid and reversible changes in the methylation status of genes, thereby regulating gene transcription, and these changes have been implicated in persistent pain conditions. In a preliminary screening, we discovered that complete Freund's adjuvant (CFA)-induced inflammation of the masseter muscle is associated with a reduction in global DNA methylation in TG, which is accompanied by a decrease in the level of DNA methyltransferase (DNMT) 1 and 3a expression. This change in global DNA methylation in TG may constitute composite responses of multiple genes. Therefore, in order to explore the role of DNA methylation on individual pro-nociceptive genes that contribute to inflammatory mechanical hyperalgesia, we used the following criteria to select candidate molecules in a relatively unbiased manner: (1) Pain-related genes that are significantly upregulated in TG following masseter inflammation from our RNAseq analysis, (2) Demonstrated in literature to contribute to inflammatory mechanical hyperalgesia arising from craniofacial muscles, (3) CpG islands can be identified through genome browser searches for the genes that qualify criteria 1 and 2, and (4) Demonstrate transcriptional modulation via specific inhibition of DNA methylation with 5-aza-dc. We identified three genes, *Trpv1, Trpa1, and P2X3,*that satisfy both criteria 1 and 2. Along with these genes, we included *Piezo2*for further confirmation. Our genome browser search showed that all four genes contain CpG islands. Treatment of TG cultures with 5-aza-dc dose-dependently increased the expression of TRPV1, TRPA1, and P2X3, but not PIEZO2. Systemic treatment of 5-aza-dc in intact animals significantly upregulated the expression of TRPV1, TRPA1, and PIEZO2 in TG. These data suggested that alterations in TRPV1 and TRPA1 expression are reliably observed with DNMT inhibition and that the expression of P2X3 and PIEZO2 is also subject to modulation by DNA methylation. The role of DNMT3a on the candidate genes was further confirmed via viral transduction of DNMT3a into TG, which led to opposite results to those of 5-aza-dc. The functional consequences of these changes are being verified by electrophysiological recordings from individual TG neurons. Future experiments will examine whether specific manipulation of DNA methylation of each gene in TG is able to alter inflammatory pain responses.

Disclosures: **J. Joseph:** None. **G. Bai:** None. **H. Ross:** None. **Y. Zhang:** None. **M. Chung:** None. **K.S. Lee:** None. **J.Y. Ro:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.05/J17

Topic: D.03. Somatosensation – Pain

Support: MOST 105-2314-B-384 -008 -MY3
EDAHP 108010

Title: The induction of inflammatory pain is associated with the downregulation of microRNA 3584 and upregulation of disintegrin and metalloprotease 19

Authors: *P.-H. TAN[1], C.-C. LIU[2];
[1]Chi-Mei Med. Ctr., Tainan, Taiwan; [2]E-Da Hospital/I-Shou Univ., Kaohsiung, Taiwan

Abstract: To understand the mechanism of inflammatory pain, it is important to investigate the development of inflammatory pain at the transcriptional and translational levels. Thus, given that microRNAs (miRNAs) are part of mechanisms of gene expression, miRNAs are expected to be involved in the regulation of proteins crucial to the pain processing pathway. To identify the biologically relevant gene targets for miRNAs, we performed an integrated analysis of both miRNA and mRNA expression profiles in the rat spinal cord during complete Freund's adjuvant (CFA)-induced inflammatory pain. We generated miRNA and mRNA arrays from the same spinal cord dorsal horn of rats on Days 5 (early phase; n=3) after CFA injection and naïve rats. Using bioinformatic analysis, the miRNA-mRNA interaction networks were studied. For validation of microRNAs, qRT-PCR was used to analyze the spinal cord sample of rats with inflammatory pain. Validation of the miR-3584-5p and ADAM (a disintegrin and metalloprotease)19 interaction was also performed (n=5). Significant changes in miR-124, miR-149, miR-3584 and their target genes, IL6R, ADAM19, LAMC1 and CERS2 were noted in the CFA 5d group. Significant downregulatin of 2.57 folds of miR-3584 and upregulation of its target gene ADAM 19 were found. Therefore, we also investigated an interaction pair, miR-3584-5p and ADAM 19, and the results showed that intrathecal administration of miR-3584-5p could attenuate inflammatory pain and decrease the expression of ADAM19. Additionally, the expression of ADAM19 target chemokine CX3C ligand-1 (CX3CL1) was decreased after administration of miR-3584-5p. The induction of CFA-induced inflammatory pain was associated with the downregulation of miR-3584 and upregulation of ADAM 19.

Disclosures: P. Tan: None. **C. Liu:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.06/J18

Topic: D.03. Somatosensation – Pain

Title: Lysophosphatidic acid mediates formalin-induced inflammatory pain in mice

Authors: *Y. HOSHINO[1], N. ITO[1], D. SAIGUSA[2], T. OKUNO[3], T. YOKOMIZO[3];
[1]Univ. of Tokyo, Department of Anesthesiology, Japan; [2]Tohoku University, Department of Integrative Genomics, Japan; [3]Juntendo Univ., Department pf Biochemistry, Japan

Abstract: Lysophosphatidic acid (LPA) evokes a variety of biological effects. Previous studies have suggested that LPA and LPA_1 receptor axis plays a major role in the pathophysiology of neuropathic pain. However, few studies have focused on the roles of LPA on acute inflammatory pain to date. To elucidate the contribution of LPA in the acute inflammatory pain, we tested the pain behavior with three ways of pharmacological inhibition of LPA, inhibition of autotaxin (ATX), which hydrolyzes LPC (lysophosphatidylcholine) to LPA, and systemic and local administration of $LPA_{1/3}$ receptor antagonist. Levels of LPA species in the hind paw were also analyzed by tandem liquid chromatography-mass spectrometry (LC-MS) and imaging mass spectrometry. <Method> C57BL6/6J male mice (8 to 13 weeks) were used. Baseline nociceptive responses following systemic administration of ATX inhibitor or $LPA_{1/3}$ receptor antagonist were assessed. Mechanical nociceptive thresholds were assessed with Von Frey filament. Thermal nociceptive thresholds were assessed with Hargreaves test. Formalin test was used to evaluate acute inflammatory pain response. To ensure scientific rigor, some experiments were conducted in a double-blind. Activation of Cyclic AMP-responsive element binding protein (CREB) in the spinal cord in response to peripheral noxious stimulation is known as an initial important step for central sensitization. Expression of phosphorylated CREB in the dorsal horn (DH) of the spinal cord following intraplantar injection of formalin were compared by immunohistochemistry between $LPA_{1/3}$ receptor antagonist group and vehicle group. The proportion of LPA species and the amount of LPA in hind paw tissues at 20 min and 2 h, following formalin injection and naive was measured using LC-MS. In addition, imaging mass spectrometry was used to identify the anatomical localization of the respective LPA species. Two-way analysis of variance (ANOVA) and Bonferroni post hoc tests were applied to the comparison between groups. <Result> Acute inflammatory pain responses were attenuated in all groups by treatments with ATX inhibitor, systemic and local administration of $LPA_{1/3}$ receptor antagonist. The expression of pCREB in DH following formalin injection was attenuated by local administration of $LPA_{1/3}$ receptor antagonist. The increase of total LPA amounts was not detected on LC-MS following formalin injection, but the localization of the respective LPA species was significantly changed. <Conclusion>These results suggest that locally produced

LPA mediates acute inflammatory pain through $LPA_{1/3}$ activation and is involved in CREB signaling which lead to the central sensitization.

Disclosures: **Y. Hoshino:** None. **N. Ito:** None. **D. Saigusa:** None. **T. Okuno:** None. **T. Yokomizo:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.07/J19

Topic: D.03. Somatosensation – Pain

Support: 17K1113 from the Japanese ministry of Education (T.I.)

Title: Is cannabinoid system involved in prostaglandin synthesis during inflammatory hyperalgesia?

Authors: *T. IBUKI[1], Y. YAMAZAKI[2], H. KITAGAWA[3], K. MATSUMURA[4];
[1]Anesthesiol., Kyoto Prefectural Univ. Med., Kyoto, Japan; [2]Anesthesiol., Kusatsu Gen. Hosp., Kusatsu, Japan; [3]Grad. Such of Engin., [4]Grad. Sch. of Engin., Osaka Inst. of Technol., Osaka, Japan

Abstract: Hyperalgesia induced by peripheral inflammation seems closely related with prostaglandin E2 (PGE2) synthesized in the central nervous system (CNS). In our previous studies, these prostaglandins seem to be produced in cyclooxygenase-2 (COX-2) dependent pathway in vascular endothelial cells in the CNS. It is widely accepted that arachidonic acid, the substrate of COX, is released from membrane phospholipid by the action of phospholipase A2. Recently, however, another pathway of releasing arachidonic acid independent of PLA2 was discovered. In this newly discovered pathway, 2-arachidonoyl glycerol (2-AG) supplies arachidonic acid. This 2-AG, an endogenous cannabinoid, is decomposed to arachidonic acid by the action of monoacylglycerol lipase. The purpose of this study was to examine the involvement of cannabinoid in the synthesis of PGE2 during inflammatory hyperalgesia. Male Lewis rats were injected with complete Freund's adjuvant (CFA) in the hind paw. Behavioral hyperalgesia was observed. Animals were sacrificed for the measurement of PGE2 in the cerebrospinal fluid and the detection of COX-2, microsomal-type PGE synthase (mPGES) and mPGES mRNA in the CNS. Further, brains were divided into 6 parts for the *ex vivo* analysis of PGE2 synthesis following incubation with NS398, a COX-2 selective inhibitor, or KT 109, an inhibitor of diacylglycerol lipase (DAGL) that is involved in 2-AG synthesis. Results showed that COX-2, mPGES protein and mPGES mRNA expression were induced in the vascular endothelial cells of the CNS by CFA. In most vascular endothelial cells, COX-2 and mPGES were co-localized. The time courses of PGE2 elevation and COX-2 expression were similar; reached the maximum on

Day 1 and then decreased. Behavioral hyperalgesia at the ipsilateral side appeared immediately, reached the maximum within 1 day, and remained elevated. In the *ex vivo* experiment, synthesis of PGE2 was inhibited by NS398 but not by KT109. We confirmed that vascular endothelial cells in the CNS play the key role in the synthesis of PGE2 during inflammatory hyperalgesia. In addition, the involvement of cannabinoids in the production of PGE2 during inflammatory hyperalgesia is less likely.

Disclosures: T. Ibuki: None. **Y. Yamazaki:** None. **H. Kitagawa:** None. **K. Matsumura:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.08/J20

Topic: D.03. Somatosensation – Pain

Support: NIH Grant F32NS106789
NIH Grant NS040538
NIH Grant NS070711

Title: TRPC5 is required for mechanical hypersensitivity in persistent inflammatory pain models

Authors: *K. SADLER, F. MOEHRING, C. L. STUCKY;
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Abstract: Many members of the transient receptor potential (TRP) superfamily are promiscuous cation channels that, when activated by ligand binding, temperature changes, or mechanical force, drive activity in peripheral sensory neurons. TRP canonical subfamily member 5 (TRPC5) is expressed in small-diameter dorsal root ganglia (DRG) neurons and is sensitive to innocuous cold temperature, membrane tension, and bioactive lipids amongst other stimuli. Global TRPC5 knockout mice exhibit normal mechanical, heat, and cold sensitivity, suggesting functional redundancy between this channel and others in naïve somatosensation. However, the lipid-sensitivity of this channel position it as a possible contributor to nociceptive signaling in persistent inflammatory pain states, many of which are characterized by lipid dysregulation. In these experiments, the requirement of TRPC5 for pain-like behaviors and nociceptive signaling was assessed using pharmacological inhibition (e.g. AC1903 compound) and genetic knockout approaches.
Intraplantar administration of AC1903 significantly reduced the punctate and dynamic mechanical hypersensitivity exhibited by Berkeley transgenic sickle cell disease (SCD) mice, a model of chronic inflammatory and neuropathic pain. Additionally, incubation with AC1903 decreased the amplitude of mechanically-evoked whole cell currents and the percentage of

rapidly adapting mechanical currents recorded in SCD DRG neurons. The requirement of TRPC5 for acute and persistent pain that was strictly inflammatory was investigated using the plantar incision and complete Freund's adjuvant (CFA) models respectively. TRPC5 knockout mice did not develop mechanical allodynia following CFA injection and returned to baseline mechanical sensitivity levels more quickly than control mice following plantar incision. The requirement of TRPC5 for neuropathic pain was investigated using the spared nerve injury (SNI) model. TRPC5 is not required for the mechanical allodynia associated with neuropathic injury as TRPC5 knockout mice and controls displayed similar decreases in von Frey withdrawal thresholds following SNI. Similarly, no differences in SNI-induced cold hyperalgesia were observed between TRPC5 knockout mice and controls. In order to identify conserved lipid agonists across the pain models in which TRPC5 was critical, lipid mass spectroscopy was performed on various biological samples. Additionally, TRPC5 expression and localization were analyzed in tissues obtained from injured mice. Based on these results, TRPC5 is required for the development of mechanical allodynia in persistent inflammatory pain states.

Disclosures: **K. Sadler:** None. **F. Moehring:** None. **C.L. Stucky:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.09/J21

Topic: D.03. Somatosensation – Pain

Support: the CAMS Innovation Fund for Medical Sciences (CIFMS)
National Science Foundation for Young Scientist of China #81801114 (F.L.)
the PUMC Innovation Fund for Postgraduate #2016 (F.L.)

Title: Neuronal Fcγ receptor type 1 contributes to antigen-induced inflammatory pain

Authors: *F. LIU, T. WANG, B. YUAN, C. MA;
Inst. of Basic Med. Sciences, CAMS&PUMC, Beijing, China

Abstract: Pain is a major symptom in certain immune-related disorders that were characterizedwith an elevated level of serum or diseased tissue IgG immune complex (IgG-IC). FcγR type I (FcγRI) is the high-affinity activating receptor of IgG-IC and critically involved in a number of inflammatory and immune diseases. Our previous studies demonstrated that a subpopulation of nociceptive dorsal root ganglion (DRG) neurons express FcγRI and can be directly activated by IgG-IC evoking an increased intracellular calcium level and neuronal excitability. This mechanism may be involved in the development of pain in multiple immune diseases such as rheumatoid arthritis. We hypothesize that IgG-IC directly activates nociceptive neurons innervating the joint by neuronal FcγRI to produce pain and inflammation in the rat

model of rheumatoid arthritis. We generate FcγRIαloxP transgenic rat line in which syntropy loxP sequence were inserted the exon both ends of genome FcγRIα gene and pirtCre transgenic rat line which the Cre protein was expressed under the control of the pirt promoter, which expressed in the primary nociceptive neurons. The FcγRIαloxP rat was crossed with pirtCre rat to generate the pirt-control conditional FcγRIα knockout transgenic rat line (Pirt-FcγRIα-/- rat). We found that the mechanical and thermal hyperalgesia induced by intracutaneous injection of IgG-IC (antigen: oval albumin, OVA, antibody: rat anti-OVA IgG) were significantly alleviated in Pirt-FcγRIα-/- rats as compared to the wildtype. We next produced a rat model of antigen-induced arthritis (AIA) using OVA as antigen, and found that both the pain-related behaviors and joint inflammation induced by AIA were significantly reduced in the Pirt-FcγRIα-/- rats as compared to the wildtype. Our results suggest that FcγRI expressed in the peripheral nociceptive neurons innervating the joint can be directly activated by IgG-IC and play an important role in the development of inflammatory pain in antigen-induced arthritis.

Disclosures: F. Liu: None. **T. Wang:** None. **B. Yuan:** None. **C. Ma:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.10/J22

Topic: D.03. Somatosensation – Pain

Support: NIH grant DA 040965
NIH grant P30GM103398

Title: Cathepsin K, a novel mediator of chronic inflammatory pain

Authors: *M. PARACHA[1], A. THAKAR[1], R. A. DARLING[2], T. E. BROWN[1];
[1]Sch. of Pharm., [2]Univ. of Wyoming, Laramie, WY

Abstract: Currently available therapeutic drugs for chronic pain only relieve the pain symptoms temporarily, have adverse effects (i.e. addiction and tolerance), and do not address the basic underlying pathology. Inhibition of the activity of cathepsin K, a cysteine protease, which has traditionally been studied in the context of osteoporosis has shown to reduce pain in the guinea pig model of inflammatory osteoarthritis (McDougall et al., 2010). However, whether cathepsin K is a key mediator of chronic inflammatory pain is not known. Previously we have shown that one-time inhibition of cathepsin K activity reduced the mechanical hypersensitivity induced by complete Freund's adjuvant (CFA) 1 day after CFA injection (control=0.53g; cKi=1.23g). These pharmacological findings were also corroborated in the cathepsin K knockout mice ($Cstk^{-/-}$). $Cstk^{-/-}$ mice showed a higher mechanical pain threshold than control mice after CFA injection (1d-post CFA: wt=0.75g; $Cstk^{-/-}$=1.47g). This data for mechanical threshold was acquired using

a von Frey filament testing method where filaments were applied in an ascending order until a response was seen for more than 5 out of 10 applications with a particular filament of specific weight. This weight was considered the mechanical threshold of the animal. Recently we have been able to reproduce the changes in the pain threshold of $Cstk^{-/-}$ and control mice with SUDO method (1d-post CFA: wt=0.60g; $Cstk^{-/-}$=1.63g). A similar reduction in mechanical hypersensitivity was also seen after inhibition of Cathepsin K activity for 2 days after CFA injection (1d-post CFA: control=0.62g, cKi=1.15g; 2d-post CFA: control=0.71g, cKi=1.33g). Furthermore, our qPCR studies show that the cathepsin K mRNA levels from CFA-injected paws, dorsal root ganglia and spinal cords are elevated by 200%-600% and western blot shows about 2.5 fold increase in cathepsin K protein in the paw of CFA-injected mouse compared to saline-injected mouse, 1 day after CFA/saline injections. From these results we hypothesize that cathepsin K is a key mediator of chronic inflammatory pain. The future goal for this work is to see whether novel strategies to attenuate cathepsin K activity can be used to treat chronic pain.

Disclosures: M. Paracha: None. **A. Thakar:** None. **R.A. Darling:** None. **T.E. Brown:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.11/J23

Topic: D.03. Somatosensation – Pain

Support: FAPESP: 2017/19105-8

Title: Release of ATP in the DRG participates of the development of inflammatory pain

Authors: *J. B. P. LEMES[1], K. F. MALANGE[1], N. S. CARVALHO[1], C. M. C. LOTUFO[2], C. A. PARADA[1];
[1]State Univ. of Campinas - Unicamp, Campinas, Brazil; [2]Univ. Federal de Uberlândia, Uberlândia, Brazil

Abstract: Introduction: Sensory ganglia are the first station in the somatosensory pathway in which pain can be modulated. Dorsal root ganglia (DRG) contain the cell bodies of primary afferent neurons, which transmit the nociceptive signal from the peripheral tissue to central nervous system. Sensory neurons do not form proper synapses at the DRG, but the neuronal soma is able to release glutamate, ATP and Substance P. One of the main mediators on the communication between neurons and satellite glial cells (SGC) in DRG is ATP. This molecule promotes excitation of the primary sensory neurons, spontaneous pain behavior, thermal and mechanical hyperalgesia. The mechanism involved on the ATP release in DRG is still poorly understood.
Aim: In this present work, we evaluate two mechanism of ATP release: 1. pannexin-1 channel,

and 2. lisossomal exocytosis.
Methods: Behavioral pharmacology study in male rats (5 weeks) was performed to evaluate inflammatory hyperalgesia (carrageenan, 100 μg/50 μL) or TRPV1+ fibers stimulation (capsaicin, 10 μg/50 μL), administrated in the intraplantar surface of hind paw. Mechanical nociceptive threshold was measured by the electronic von Frey method for carrageenan test, and flinches of stimulated paw was counted for 5 minutes after capsaicin. Immunofluorescence assay was performed to analyze the site of the ATP stocks in the DRG-L5. Quinacrine (ATP marker) was co-marked with TRPV1 or NF200 antibodies.
Results: Pre-treatment (30 minutes before) of ^{10}Panx (Panx1 blocker) or Vacuolin-1 (lysossomal exocytosis inhibitor) in ipsilateral DRG-L5 reduced both carrageenan-induced hyperalgesia and capsaicin-induced nociception. Data of imunofluorescence showed the presence of stocks of ATP in the small (TRPV1+), medium and large (NF200+) DRG neurons.
Discussion and Conclusion: The results suggested that ATP releasing in DRG is important to development of inflammatory pain and the nociception mediated by C-fibers.

Disclosures: J.B.P. Lemes: None. **K.F. Malange:** None. **N.S. Carvalho:** None. **C.M.C. Lotufo:** None. **C.A. Parada:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.12/J24

Topic: D.03. Somatosensation – Pain

Title: Satellite glial cells are required for painful inflammatory response

Authors: *J. LAPP[1], K. E. MILLER[2];
[1]Oklahoma State University- Ctr. For Hlth. Scien, Tulsa, OK; [2]Dept Anat. & Cell Biol, Oklahoma State Univ. Ctr. for Hlth. Sci., Tulsa, OK

Abstract: Introduction: Glutamine Synthetase (GS) is an enzyme important for the degradation of neurotransmitter glutamate to glutamine. Satellite glial cells (SGCs) surround dorsal root ganglion (DRG) neurons and have been observed to contain abundant levels of GS. DRG neurons are known to alter concentrations of glutaminase (synthetic enzyme for neurotransmitter glutamate) during peripheral inflammation and subsequent sensitization, but potential complimentary alterations in GS levels are unknown. If GS or SGC function is disrupted, it may lead to aberrant pain processing and dysregulation of glutamate.
Aim: The current study was undertaken to determine if GS mRNA and/or protein levels are altered in rat DRG during acute peripheral inflammation.
Methods
Peripheral inflammation was induced via injection of Complete Freund's Adjuvant (CFA) in

right hindpaw of anesthetized, albino Sprague Dawley rats. Western blot, qPCR, and immunohistochemistry were performed to see expression profile of GS in DRG neurons at 24h and 48h. In addition, glutamate metabolic inhibitors were added to the peripheral injection under the same conditions and compared to CFA-only animals.

Results

mRNA and Protein results in experimental animals show GS levels increase at day one, and continue to increase further at day two. DRG Immunofluorescence data are consistent with these results.

Conclusion

These data further our understanding of the glutamate/glutamine cycle and of the communication between SGC's and DRG neurons in relation to nociception and inflammation.

Disclosures: J. Lapp: None. **K.E. Miller:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.13/J25

Topic: D.03. Somatosensation – Pain

Title: Peripheral antinociceptive effect of oleanolic acid in a rat model of joint inflammation

Authors: *I. M. SALMAN[1], M. FOUANI[1], W. NAJJAR[1], N. LAWAND[1,2];
[1]Dept. of Anatomy, Cell Biol. and Physiological Sci., [2]Dept. of Neurol., American Univ. of Beirut, Beirut, Lebanon

Abstract: Knee joint inflammation causes hypersensitivity and pain to thermal and mechanical stimulation, and triggers a prolonged increase in the excitability and synaptic efficacy of central nociceptive neurons. Oleanolic Acid (OA) is a naturally occurring pentacyclic triterpenoid present in food and in plants. It has a neuroprotective role on the neurons of the central nervous system. In this study, we explored whether a pretreatment of OA injections intra-articularly alter peripheral nociception.

Sensory and motor tests were performed prior to, and at 4, 8, 24, 48hrs and 1wk post induction of inflammation. The joint circumference was measured to evaluate the development of edema in all rats. The rats were divided into two groups, one receiving OA and one receiving ethanol before the induction of inflammation. The sensory and the motor tests were analyzed, monitored and followed in order to spot any differences in the performances of the rats.

Symptoms of inflammation were manifested in all rats injected with K/C. Rats receiving OA showed a noticeable decrease in hypersensitivity when subjected to sensory or motor stimuli as compared to rats receiving ethanol. Consequently, intra-articular injections of OA showed peripheral anti-nociceptive effects not exhibited with ethanol injections, this portrays potential

benefits of OA as an efficient and effective pretreatment for the management of peripheral nociception induced by knee joint inflammation.

Disclosures: I.M. Salman: None. **M. Fouani:** None. **W. Najjar:** None. **N. Lawand:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.14/J26

Topic: D.03. Somatosensation – Pain

Title: FAM19A1, a chemokine-like secreted protein, is required for mechanical allodynia

Authors: *H. JIANG, G. YU, C. GUO, M. XIAO, L. ZHANG, D. KRANZ, W. YANG, F. LI, Q. LIU;
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Abstract: Mechanical allodynia is the most common symptom in both inflammatory and neuropathic conditions. However, effective treatments for mechanical allodynia are lacking. FAM19A1 (family with sequence similarity 19, member A1, also called TAFA1) is a chemokine-like secreted protein with unknown function. Using X-gal staining and the FAM19A1-LacZ reporter mice, we found that FAM19A1 is extensive expressed in myelinated primary sensory neurons. Furthermore, the retrograde labeling showed that FAM19A1^{+} DRG neurons innervate both the hairy skin and glabrous skin. Although FAM19A1-null mice showed normal acute pain responses, they displayed significantly attenuated mechanical allodynia albeit normal thermal hyperalgesia or cold allodynia in chronic inflammation. Remarkably, intrathecal injection of FAM19A1 induced mechanical allodynia but not thermal hyperalgesia or cold allodynia in WT mice. Our data may provide a new target for the treatment of mechanical allodynia under pathological conditions.

Disclosures: H. Jiang: None. **G. Yu:** None. **C. Guo:** None. **M. Xiao:** None. **L. Zhang:** None. **D. Kranz:** None. **W. Yang:** None. **F. Li:** None. **Q. Liu:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.15/J27

Topic: D.03. Somatosensation – Pain

Support: The Royal Golden Jubilee Scholarship

Title: The development of bilateral pain hypersensitization in a mouse model of chronic temporomandibular disorder induced by unilateral CFA injection

Authors: N. ROTPENPIAN[1], A. KAEWPITAK[2], A. WANASUNTRONWRONG[1], ***N. PAKAPROT**[1];
[1]Mahidol Univ., Bangkok, Thailand; [2]Prince of Songkla Univ., Songkla, Thailand

Abstract: Temporomandibular disorder (TMD) is a major dental condition, causing a chronic widespread pain in the orofacial area. It is a result of multiple factors at the temporomandibular joint (TMJ) e.g. trauma or occlusal changes with tissue degeneration. The pathophysiology of chronic TMD induced orofacial pain is still unclear, resulting in non-specific treatments and unpredictable outcomes. The researchers have tried to develop models of the TMD induced orofacial pain and found that chemical substances infused into the TMJs can cause pain similar to TMD induced pain. The present study aimed to investigate the development of pain hypersensitization after chronic TMD pain induced by CFA injection. Complete Freund's adjuvant (CFA) is a solution of inactive mycobacteria antigen emulsified in mineral oil, and it can cause inflammation in the injected tissue. The 6 weeks old ICR male mice were divided into 2 groups, sham and CFA (n=9). The mice were injected into the right TMJ with either normal saline (10 µl of 0.9% normal saline) or CFA (10 µl of 50% CFA) for the sham and CFA groups, respectively. Then, the mice were tested for the development of pain hypersensitization by Von-Frey, air-puff and cold acetone tests to investigate the presence of mechanical hyperalgesia, mechanical allodynia and cold allodynia at day 3, 7, 14, 21 and 28 after the injection, respectively. In addition, the TMJs were collected to examine the bone structure and inflammation around the TMJs by hematoxylin and eosin staining, and micro-CT scan at day 28 after the injection. The results revealed that the CFA induced TMD mice significantly developed mechanical allodynia, cold allodynia and mechanical hyperalgesia ipsilateral to the site of CFA injection at day 14, 21 and 28 when compared to the sham group ($P<0.05$). The pain hypersensitization on the ipsilateral side corresponded with significant changes in bone structures caused by the inflammation around the injected TMJs in the CFA group. Moreover, all of the abnormal pain responses (mechanical allodynia, cold allodynia and mechanical hyperalgesia) have also developed on the contralateral side at day 28 compared to the sham group. Therefore, the inflammation induced by CFA injection could cause TMD, leading to the development of pain hypersensitization in the orofacial region similar to the patients with chronic TMD. The presence of pain hypersensitization on the contralateral side also indicated the development of neuropathic pain, suggesting the involvement of neuropathology occurred after the prolonged TMD. In summary, this study supported the benefit of CFA injection model as a good model for the investigation of chronic TMD induced orofacial pain.

Disclosures: N. Rotpenpian: None. **A. Kaewpitak:** None. **A. Wanasuntronwrong:** None. **N. Pakaprot:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.16/J28

Topic: D.03. Somatosensation – Pain

Title: The expression of aspartate aminotransferase and glutaminase in dorsal root ganglion of rats with genetically induced hypoglutamatergic tone during peripheral inflammation

Authors: *R. D. PANDE, K. E. MILLER;
Anat. and Cell Biol., Oklahoma State Univ. Ctr. For Hlth. Sci., Tulsa, OK

Abstract: Most dorsal root ganglion (DRG) neurons are glutamatergic utilizing phosphate-activated glutaminase (GLS) and cytosolic aspartate aminotransferase (cAST) for the synthesis of major excitatory neurotransmitter glutamate. Glutamate as neurotransmitter derives mainly from glutamine-glutamate cycle via GLS. Our previous finding showed elevated levels of GLS in DRG neurons during peripheral inflammation. Although cAST can provide a net synthesis of glutamate, little is known about its precise functional role in sensory neurons during inflammation. In addition, the incomplete understanding of the interplay between GLS and cAST led us to investigate the expression of these enzymes in rats with genetically induced hypoglutamatergic tone (Heterozygous: GLS+/-). Heterozygosity of the GLS1+/− rats was verified by genotyping using polymerase chain reaction. Antigen-induced arthritis (AIA) was induced by injecting complete Freund's adjuvant (CFA) into the right hind paw of anesthetized, 8-10-week-old male WT and GLS+/- Sprague Dawley rats (200-300gm). L4 and L5 DRG were collected from control and AIA animals at 24 and 48 hours of inflammation. Messenger RNA and protein expression of cAST and GLS in DRG was determined by quantitative PCR and immunoblot techniques, respectively. Our findings show an increase in the expression of cAST and GLS in the DRG of WT rats indicating a change in glutamate synthesis during peripheral inflammation. However, the change in expression of these enzymes in GLS+/- rats were less significant representing the probable interplay between GLS and cAST during peripheral inflammation.

Disclosures: **R.D. Pande:** None. **K.E. Miller:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.17/J29

Topic: D.03. Somatosensation – Pain

Support: CNPq - National Council for Scientific and Technological Development
CAPES - Committee for the Development of Higher Education Personnel
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Grant 150530
PROPESq/UFRGS - Drug Discovery CAPES – University of Nottingham Grant 41/2014
FAPERGS DocFix/CAPES 04/2018

Title: IB-MECA acute treatment relieves pain in CFA chronic inflammatory model in rats

Authors: *J. A. F. ASSUMPÇÃO[1], S. G. CIOATO[2,3], L. F. MEDEIROS[2,3,4], B. C. LOPES[1,3], A. A. SALVI[1,3], A. SOUZA[1,5], R. ROESLER[1], W. CAUMO[1], I. L. S. TORRES[1,3];
[2]Dept. de Farmacologia, [1]UFRGS - Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil; [3]HCPA - Hosp. de Clínicas de Porto Alegre, Porto Alegre, Brazil; [4]Unilassale - Ctr. Universitário Unilas, Canoas, Brazil; [5]Unilassale - Ctr. Universitário Unilasalle, Canoas, Brazil

Abstract: It is known that IB-MECA compound, an agonist of adenosine A3 receptor (A3AR), is involved with pain relief and modulation in the inflammatory process; however, its action mechanisms are not completely elucidated. The aim of this study was to evaluate the antinociceptive effect of IB-MECA) in a chronic inflammatory pain model, and the involvement of neurotrophins and cytokines central levels in this effect. Chronic inflammatory pain was induced using Complete Freund's Adjuvant (CFA) in the hind paw of male adult Wistar rats. Thermal and mechanical hyperalgesia/allodynia was measured by Hot plate, Von Frey and Randal Selitto tests. Neurochemical measured were brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), interleukin 1β (IL-1β) and IL-10. The establishment of pain model, decrease of latency withdrawal, was observed 10 and 14 days after CFA injection. And, IB-MECA was effective to revert mechanical and thermal hyperalgesia, in a totally or partially way. We observed CFA pain model effects in IL-1β and IL-10 spinal cord and brainstem levels. Also,we showed that IB-MECA administration in controls increased the interleukin levels. And, we did not find any involvement neurotrophins in this effect, at least those we measured in spinal cord and brainstem of rats. Studies have shown that CFA increases the IL-1β in the hind paw of injection when evaluated acutely after the induction of model. But, there is a lack of knowledge about the neuroinflammatory effects induced by CFA pain model. Also, the adenosine A3

receptor seems to have complexes effects in the central nervous system, with proinflammatory and anti-inflammatory roles, specially in healthy conditions.

Disclosures: J.A.F. Assumpção: None. **S.G. Cioato:** None. **L.F. Medeiros:** None. **B.C. Lopes:** None. **A.A. Salvi:** None. **A. Souza:** None. **R. Roesler:** None. **W. Caumo:** None. **I.L.S. Torres:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

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Program #/Poster #: 056.18/J30

Topic: D.03. Somatosensation – Pain

Support: NIH RO1 AT009366
Dept of the Army Log No. 14001001
R01 DA044934-02

Title: The effect of morphine on post-operative allodynia and toll like receptor 4 mediated inflammation in male and female rats

Authors: *M. E. HARLAND[1], J. B. BALL[1], A. J. KWILASZ[1], S. M. FULGHAM[1], R. A. DREHER[1], S. F. MAIER[1], K. C. RICE[2], P. M. GRACE[3], L. R. WATKINS[1];
[1]Psychology and Neurosci., Univ. of Colorado, Boulder, CO; [2]Intramural Res. Program, Natl. Inst. on Drug Abuse, IRP, NIH, Baltimore, MD; [3]Dept. of Symptom Research,, Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Our lab has previously shown that a short course of morphine potentiates neuropathic pain in the chronic constriction injury (CCI) model in male and female rats and in a post-operative (i.e. laparotomy) model of pain in male rats. We have further demonstrated that the potentiation of neuropathic pain by morphine is mediated by activation of toll like receptor 4 (TLR4) and downstream activation of the Nod-like receptor protein 3 (NLRP3) inflammasome pathway, which causes release of pro-inflammatory cytokines that mediate pain such as interleukin-1βin male rats. Whether female rats express the same morphine-induced TLR4 activation pathway to the NLRP3 inflammasome in either neuropathic pain or postoperative pain is unknown. In the experiments presented here we have explored the prolonged allodynia following morphine treatment in the post-operative pain model of paw incision, and how this is altered by inhibition of TLR4 by the co-administration of the TLR4 antagonist (+)-naloxone (20mg/kg) along with a 5-day course morphine (5mg/kg) in male and female rats.

Disclosures: **M.E. Harland:** None. **J.B. Ball:** None. **A.J. Kwilasz:** None. **S.M. Fulgham:** None. **R.A. Dreher:** None. **S.F. Maier:** None. **L.R. Watkins:** None. **P.M. Grace:** None. **K.C. Rice:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.19/J31

Topic: D.03. Somatosensation – Pain

Support: NIH RO1 AT009366
Dept of the Army Log No. 14001001
Univ. of Texas start up funds to Peter Grace

Title: Six weeks of prior voluntary exercise induces a sex dependent antioxidant response which is protective after chronic constriction injury

Authors: *S. M. FULGHAM[1], J. B. BALL[1], M. E. HARLAND[1], R. A. DREHER[1], A. J. KWILASZ[1], S. F. MAIER[1,2], L. R. WATKINS[1], P. M. GRACE[2];
[1]Psychology & Neurosci., CU Boulder, Boulder, CO; [2]Symptom Res., Univ. of Texas MD Anderson, Houston, TX

Abstract: As research has expanded to increasingly include both males and females in experimental groups, sex differences often emerge. Our lab has previously shown that 6 weeks of prior voluntary wheel running (ending at the time of nerve injury) prevents the development of neuropathic pain following chronic constriction injury (CCI) in male rats. We have found that this effect replicates in female rats. The mechanism by which exercise is protective is multifaceted and complex. Here we explored one possible mechanism; that is, the exercise induced antioxidant response. We hypothesize that the antioxidant response induced by the oxidative stress of exercise plays a role in the attenuation of allodynia following CCI in rats with prior exercise, as accumulation of reactive oxygen and nitrogen species after peripheral nerve injury can promote neuropathic pain. The cellular mechanisms under investigation have revealed various sexually dimorphic effects in the response to voluntary exercise. In the experiments presented here,we have first explored the antioxidant response evoked in dorsal lumbar spinal cord and sciatic nerve by 6 weeks of voluntary wheel running exercise in uninjured male and uninjured female rats. We investigated the effects of exercise on the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and several antioxidant enzymes known to be upregulated by this transcription factor, superoxide dismutase (SOD1 and SOD2),and catalase. Our data demonstrate that 6 wk of prior voluntary wheel running induced greater antioxidant response in females than males. Secondly, we quantified the changes in antioxidant response and a marker of nitroxidative damage, nitrotyrosine, occurring in these same tissues at 1 week and 2 weeks post

CCI in males and females and how this was modulated by prior exercise. We found that females responded to CCI with greater antioxidant signaling and markers of oxidative stress than males in the lumbar spinal cord, whereas males showed greater response than females in the sciatic injury site. Our data show that in female rats with prior exercise, the markers of antioxidant enzymes at the injury site were still significantly higher than sedentary controls at 2 weeks post CCI. These data may indicate that although prior voluntary exercise is protective in both males and females, the antioxidant response it produces may underlie the protective effect more strongly in females more than males.

Disclosures: S.M. Fulgham: None. **J.B. Ball:** None. **M.E. Harland:** None. **R.A. Dreher:** None. **A.J. Kwilasz:** None. **S.F. Maier:** None. **L.R. Watkins:** None. **P.M. Grace:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: W81XWH-11-1-0672. US Department of Defense, Grant# 10669042
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Cleveland Clinic Anesthesiology Institute Interval Research Fund

Title: New insight of the role of FKN/CX3CR1 signaling in a mouse model of neuropathic pain

Authors: Q. FAN, F. LI, L. LIU, J. LI, Y. YIN, *J. CHENG;
Cleveland Clin., Cleveland, OH

Abstract: Objective: Neuroinflammation has been specifically implicated in neuropathic pain. There is increasing evidence that a number of cytokines and their receptors are involved in the processes that lead to the development of neuropathic pain. Fractalkine receptor (CX3CR1) is expressed constitutively in microglia in the CNS and macrophages in the PNS. It is a unique chemokine receptor that binds only to the chemokine, fractalkine (FKN or CX3CL1). We have shown that $CX3CR1^{+}$ cells play a key role in development of neuropathic pain. However, the role of FKN/CX3CR1 signaling in the pathogenesis of neuropathic pain is far from clear. In this study, we employed two fluorescein protein knock in mouse models, $CX3CR1^{GFP/+}$ and $CX3CR1^{GFP/GFP}$ mice, to investigate if blocking FKN/CX3CR1 signaling can decrease the production of the key proinflammatory cytokines. **Methods:** With IACUC approval, chronic constriction injury (CCI) of the right sciatic nerve was induced by loose ligation on $CX3CR1^{GFP/+}$ and $CX3CR1^{GFP/GFP}$ transgenic mice. Sham-operated animals (sciatic nerve exposure without ligation) were used as controls. Paw withdrawal thresholds were evaluated on post-surgical days 0, 7, 14, 21 and 28. The animals were sacrificed and perfused at these time

intervals to collect samples of the sciatic nerve (SN), DRG, and spinal cord (SC) of both sides for immunohistochemistry. Proteins of SN and SC of both sides were extracted from mice on post-surgical day 0, 3 or 7 to perform ELISA analysis using mouse Cytokine Array / Chemokine Array 31-Plex (MD31). **Results:** 1) The number and morphology of $CX3CR1^{+}$ cells were dramatically increased in SN, DRG and SC starting from post-surgical day 3 and peaked at the day 14, in CCI model of *CX3CR1*$^{GFP/+}$ mice in sync with hyperalgesia. 2) The cytokines TNFα, Eotaxin, G-CSF, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IP-10, KC, LIF, LIX, MCP-1, M-CSF, MIP-1α, MIP-1β, MIP-2, RANTES and VEGF were gradually increased in SN and SC of CCI model of *CX3CR1*$^{GFP/+}$ mice in sync with hyperalgesia. 3) Most cytokines mentioned above were more dramatically increased in *CX3CR1*$^{GFP/GFP}$ mice compared to that of *CX3CR1*$^{GFP/+}$ mice. 4) On post-surgical day 28, the number and morphology of $CX3CR1^{+}$ cells were dramatically increased in SC in CCI model of *CX3CR1*$^{GFP/GFP}$ mice compared to that of *CX3CR1*$^{GFP/+}$ mice. **Conclusion:** These data suggest that $CX3CR1^{+}$ cells in both CNS and PNS play a role in development of neuropathic pain in mice. Several key proinflammatory cytokines were not reduced by blocking FKN/CX3CR1 signaling, suggesting that the role of this signaling pathway is much more complicated than previously thought.

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Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

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Program #/Poster #: 056.21/J33

Topic: D.03. Somatosensation – Pain

Support: NIH RO1 AT009366
Dept of the Army Log No. 14001001
R01 DA044934-02

Title: Sexual dimorphism in the inflammatory response to chronic constriction injury in rats

Authors: *J. B. BALL[1], S. M. FULGHAM[1], M. E. HARLAND[1], R. A. DREHER[1], M. G. FRANK[1], S. F. MAIER[1], P. M. GRACE[2], L. R. WATKINS[1];
[1]Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO; [2]Dept. of Symptom Res., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Much of our understanding of the inflammatory response following chronic constriction injury was established in male rodents. As females are increasingly being studied in chronic pain models, sexually dimorphic effects are beginning to emerge in the inflammatory response and development of chronic pain, yet much remains poorly understood. Prior work has shown that the peripheral macrophage NOD-like receptor protein 3 (NLRP3) inflammasome

pathway established in males is not required for female allodynia in post-operative. Other studies suggest that the female response to inflammation and pain may be T cell mediated, whereas the male response may be microglial mediated. It is known that the inflammatory cytokine interleukin 1beta (IL-1beta) increase contributes to chronic pain after injury in males and females. However, the specific inflammasome(s) responsible for IL-1beta production in females vs. males is/are unknown. Our data show that IL-1beta is elevated in both males and female rats after CCI in a peripheral vs. CNS manner. That is, males show a greater increase of IL-1beta and inflammatory signaling at the sciatic injury site, whereas females show greater increase of IL-1beta and inflammatory signaling in the lumbar spinal cord. Males also show greater T cell infiltration in the injury site after CCI, whereas females show greater T cell infiltration in the spinal cord. Additionally, our data demonstrate that NLRP3 expression is male specific in the injury site in the CCI model of chronic pain, yet females show greater NLRP3 expression in the dorsal lumbar spinal cord than males after injury. The sex differences presented here in the dorsal lumbar spinal cord and sciatic nerve injury site indicate that males and females may have divergent pathways in the inflammatory sequalae underlying chronic pain development following chronic constriction injury.

Disclosures: J.B. Ball: None. **S.M. Fulgham:** None. **M.E. Harland:** None. **R.A. Dreher:** None. **M.G. Frank:** None. **S.F. Maier:** None. **L.R. Watkins:** None. **P.M. Grace:** None.

Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.22/J34

Topic: B.04. Ion Channels

Title: *Ex vivo* human models of extracellular acidification in inflammatory pain states for enabling translational research and drug discovery

Authors: *A.-T. TON, T. INDERSMITTEN, P. RATCHADA, K. SWEAT, G. PAGE, P. MILLER, A. GHETTI;
Anabios Corp., San Diego, CA

Abstract: The incomplete understanding of chronic neuropathic and inflammatory pain mechanisms creates major challenges in the efforts to develop new, non-addictive pain medications. Compounding the problem, the translation from preclinical pain animal models to humans has been unreliable at best. Indeed, cross-species difference in pharmacological response and analgesic effects are now well documented and extremely common. To address these issues, we have developed a novel preclinical discovery strategy, which relies on human sensory neurons isolated from organ donors, allowing *ex vivo* studies of sensory neurons' physiology and pharmacology. In the current study, we describe the use of isolated and cultured human sensory

neurons to establish cellular models of pathological states that recapitulate key features of inflammation-related pain and inflammation-related tissue acidification. Following treatment of the cells with agents involved in the inflammatory response, we assessed neuronal excitability by employing electrophysiology-based patch-clamp method in current-clamp mode. Treatment of human sensory neurons for 72 hours with inflammatory agents (PGE_2 and bradykinin) resulted in changes in the excitability and nociceptive properties of the cells. Meanwhile, efficacy of analgesic compounds was reduced following extracellular acidification of inflamed human sensory neurons. Indeed, these observations are consistent with the clinical manifestations of reduced analgesic efficacy under condition of inflammation-driven tissue acidification. Finally, we demonstrated that this cell-based model can be used to differentiate the effects of analgesic drugs that have demonstrated varying degree of success in clinical trials with patient populations affected by different pain indications. This approach shows high potential for ranking drug candidates based on potential analgesic efficacy and for predicting the most appropriate pain condition for clinical trial development.

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Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

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Program #/Poster #: 056.23/J35

Topic: D.03. Somatosensation – Pain

Support: FAPESP: 2017/07162-7

Title: Interaction among COX2 enzyme and NMDA, P2X7 receptors in DRG contributes to inflammatory hyperalgesia in the peripheral tissue

Authors: *N. S. CARVALHO[1], F. H. FARIAS[1], G. F. CATROLI[1], G. G. DOS SANTOS[2], A. F. NEVES[1], S. F. MAGALHÃES[1], J. B. P. LEMES[1], C. A. PARADA[1];
[1]Univ. Estadual De Campinas - UNICAMP, Campinas, Brazil; [2]Univ. of California, San Diego, CA

Abstract: Introduction: Inflammatory process releases a range of inflammatory mediators which leads to pain-producing stimuli detected by the nerve terminals of primary sensory neurons, whose cell bodies are located in the dorsal root ganglion (DRG). In this context, the interaction between neurons and satellite glial cells has been focus of several studies that seek to better understand the development of inflammatory hyperalgesia in the peripheral tissue.
Aim: Investigate whether NMDA receptor (NMDAr) activation mediates a crosstalk between neurons and satellite glial cells, and if it is dependent on P2X purinergic receptor 7 (P2X7)

and/or COX-2 activation.
Methods: Male Wistar rats (7 weeks) were treated with ganglionar (gl.) administration of oligodeoxynucleotide antisense (ODN-AS) against NMDAr – group I and II (20 µg/3 µl) or COX-2 - group III (30 µg/3 µl), or P2X7 - group IV (35 µg/3 µl) or saline group - V for 4 days. After 16 hours was administered respectively, IL-1β - group I (0.5 pg/ 3 µl/ gl.), BzATP - group II (100 nM/3 µl/ gl.) and NMDAr agonist (240 ng/3 µl/ gl.) for the group III and IV. Mechanical hyperalgesia was evaluated by electronic von Frey test. Statistical analysis were performed by one-way ANOVA followed by Tukey's post hoc test; (n = 6).
Results: The decrease in the expression of COX-2 in DRG resulted in attenuation of the mechanical hyperalgesia induced by NMDAr agonist, however, the knockdown of NMDA receptor in DRG cells, did not affect the mechanical hyperalgesia induced by IL-1β. The knockdown of NMDAr in the DRG, did not affect the mechanical hyperalgesia induced by BzATP, however, the knockdown of P2X7, prevented the mechanical hyperalgesia induced by NMDAr agonist.
Discussion and conclusion: Our results indicate an interaction among COX-2 and NMDA/P2X7 receptors in DRG cells and we suggest that this pathway is triggered by NMDA receptor activation, releasing ATP, which activates P2X7 receptors, inducing the release of IL-1β, which activates COX-2 which produces and releases PGE_2 in DRG cells, which sensitizes primary afferent nociceptors.

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Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.24/J36

Topic: D.03. Somatosensation – Pain

Support: National Heart, Lung and Blood Institute (HL135895-18)

Title: Contribution of cutaneous COX-2 to inflammation and pain in a murine model of sickle cell disease: A potential therapeutic strategy for resolvins

Authors: *B. D. MCADAMS[1], I. A. KHASABOVA[1], J. J. GABLE[1], S. G. KHASABOV[1], V. S. SEYBOLD[1], K. GUPTA[2], W. R. KENNEDY[3], A. KALYUZHNY[4], D. A. SIMONE[1]; [1]Diagnos. Biol., [2]Med., [3]Neurol., Univ. Minnesota, Minneapolis, MN; [4]R&D SYSTEMS, Minneapolis, MN

Abstract: Sickle cell disease (SCD) is the most common monogenic hemoglobin disorder characterized by oxidative stress, chronic inflammation and pain. Previously we demonstrated

that prostaglandin E2 glyceryl ester (PGE_2-G) contributes to hyperalgesia in humanized murine model of SCD through activation of $P2Y_6$ receptors (Khasabova et al., 2019). Considering that PGE_2-G biosynthesis is mediated by cycloxygenase-2 (COX-2), the expression and localization of COX-2 protein was analyzed immunohistochemically in glabrous hindpaw skin from sickle HbSS-BERK and control HbAA mice. Enhanced COX-2 immunoreactivity in dendritic cells, resembling melanocytes and Langerhans cells was shown in epidermis of hyperalgesic HbSS mice. The cells expressing COX-2 were distributed in the epidermis where nociceptive epidermal nerve fibers (ENFs) are normally present. Consistent with our previous work (Kohli et al., 2010), ENFs are largely reduced in the epidermis of HbSS mice but retained in HbAA mice. The absence of ENFs is a hallmark of peripheral neuropathies. Further evidence of systemic inflammation in HbSS mice was demonstrated by increased number of Iba1 and CD68-immunopositive cutaneous cells. Resolvins, specialized endogenous pro-resolving lipid mediators, were shown to decrease inflammation and organ damage in sickle mice exposed to hypoxia/reoxygenation (Matte et al., 2019). Since COX-2 mediates biosynthesis of PGE_2-G, and RvD1 inhibited COX-2 activity in spinal cord tissue, we therefore investigated effects of resolvin D1 (RvD1) on hyperalgesia in sickle mice. A single intraplantar injection of RvD1 (0.3 ng/10 µl, i.pl.) decreased mechanical hyperalgesia in HbSS mice. The antihyperalgesia was bilateral, occurred 20 h after injection, and persisted during the next 24 hours, suggesting a systemic mechanism of action. Thus, RvD1 may be an effective therapeutic strategy for pain in SCD patients.

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Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 056.25/J37

Topic: D.03. Somatosensation – Pain

Support: CIC UMSNH 26.10
CIC UMSNH 30.2

Title: Oral coadministration of metformin and topiramate in rat formalin test

Authors: *L. F. ORTEGA-VARELA[1], C. CERVANTES-DURAN[4], J. G. TORRES-ALVARADO[2], E. BENITEZ-FAJARDO[2], M. Y. GAUTHEREAU-TORRES[3];
[1]Escuela de Enfermería y Salud Pública, [2]Facultad de Quimico Farmacobiologia, [3]Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Univ. Michoacana de San Nicolas De Hidalgo, Morelia, Mexico; [4]Licenciatura en Tecnologías para la Información en Ciencias, Escuela Nacional de Estudios Superiores, Univ. Nacional Autonoma de Mexico, Morelia, Mexico

Abstract: Pain remains as a global public health challenge. Combination therapy is a well-established pharmacotherapeutic strategy for the treatment of various clinical disorders. This study was achieved to assess the interaction between metformin and topiramate orally administered in the rat model of formalin test. Female Wistar rats (220-350 g) were injected into the dorsal surface of the right hind paw with 50 μ g of 1% formalin. This substance induced a flinching pain-related behavior, the reduction of such conduct is considered as antinociception. The percent of antinociceptive effect was determined by the oral administration of metformin (30-1000 mg/kg), topiramate (12.5-100 mg/kg), and their combination. To analyze the nature of the interaction, isobolographic analysis was used in a fixed-dose radio (0.5:0.5), on the basis of their ED50 values: metformin (908.63 ± 280.87 mg/kg) and topiramate (44.02 ± 15.10 mg/kg). The metformin-topiramate combination significantly reduced the number of flinches in the second phase of the formalin test. For the isobolographic analyses, the theoretical effective dose 50 for the combination (ED50 T) was 476.33 ± 140.64 mg/kg. Experimentally, the effective dose 50 (ED50 E) was significantly lower (331.78 ± 76.35 mg/kg), indicating the presence of synergism for the combination. Results show that the oral coadministration of metformin and topiramate can interact synergistically and could provide a therapeutic alternative for inflammatory pain.

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Poster

056. Pain: Inflammatory Mechanisms

Location: Hall A

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Program #/Poster #: 056.26/J38

Topic: D.03. Somatosensation – Pain

Support: A3 Foresight Program from the Japan Society for the Promotion of Science

Title: Small animal neuroimaging analysis of visceral pain matrix in an inflammatory bowel disease model rats

Authors: *Y. L. CUI[1], T. HUANG[1,2,3], D. HU[1], Y. WU[1], M. SHIGETA[1], E. HAYASHINAKA[1], Y. WADA[1], K. NOGUCHI[2], Y. DAI[3], Y. WATANABE[1];
[1]RIKEN Ctr. for Biosystems Dynamics Res., Kobe, Japan; [2]Hyogo Coll Med., Nishinomiya / Hyogo, Japan; [3]Hyogo Univ. of Hlth. Sci., Kobe, Hyogo, Japan

Abstract: Inflammatory bowel disease (IBD) is a chronic inflammatory disorder characterized by swollen and damaged tissues in the digestive tract and mainly comprises Crohn's disease and ulcerative colitis. A majority of patients experience abdominal pain during acute IBD episodes, which severely impair their quality of life. Both peripheral and central mechanisms are thought to be involved in such abdominal pain in IBD. Although much attention has been paid on peripheral mechanisms of abdominal pain in IBD pathophysiology, the involvement of supraspinal mechanisms remains poorly understood. To address this issue, we investigated regional brain activity induced by noxious colorectal distension (CRD) in an IBD rat model using a small-animal neuroimaging method combining 2-deoxy-2-[^{18}F] fluoro-D-glucose PET imaging and voxel-based statistical analysis, in which 2,4,6-trinitrobenzene sulfonic acid, a chemical compound widely used to generate colitis, was applied to the colon. We show here that the the regional brain activity in normal rats increased in widespread cortical areas in response to noxious CRD, such as in the motor/somatosensory cortices, insular cortex, central amygdaloid nucleus, and so on. Meanwhile, significant increment of regional brain activity was seen in the anterior cingulate cortex, motor cortex and nucleus accumbens in the IBD model rat. These results indicate that the nociceptive pathways under persistent colorectal inflammation may be different from the physiological "pain matrix" associated with noxious mechanical stimulation.

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Poster

056. Pain: Inflammatory Mechanisms

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Program #/Poster #: 056.27/J39

Topic: D.03. Somatosensation – Pain

Support: NRF2018R1A5A2024418
NRF2017M3C7A1025602
NRF2016M3A9B6021209

Title: Acute fasting and refeeding alleviate pain via different pathway

Authors: *J.-Y. LEE[1], S. OH[2], Y. KANG[3];
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Abstract: Pain is considered to be both sensory and emotional experience. Since emotion is affected by homeostasis such as temperature, hunger, satiety and thirsty, the perception of pain is likely to be regulated by homeostatic control. In the present study, we examined whether pain perception is affected by fasting and refeeding behavior from adult mice. We found that 24h acute fasting suppressed formalin-induced paw licking behavior in the second phase and this analgesic effect still remained even after 2h of refeeding after 24h fasting. However, after 24h of refeeding, this acute fasting-induced analgesic effect disappeared. Formalin-induced c-Fos expression in the superficial dorsal horn was also suppressed by both fasting and refeeding. Intraperitoneal (i.p.) administration of CB1 receptor antagonist (SR 141716, 10 mg/kg) and opioid receptor antagonist (naloxone, 10 mg/kg) inhibited 24h fasting-induced analgesic effect, but did not affect 2h refeeding-induced analgesia. 2h refeeding with calorie-free agarose produced an additional analgesic effect compared with 24h fasting. Besides, administration of glucose by intraperitoneal (i.p.) injection, which mimics the effect of calorie recovery in refed state, induced a greater analgesic effect than fasted mice. Taken together, our results suggest that fasting and refeeding produce analgesia through different mechanisms. Endocannabinoid and opioid system is only involved in fasting-induced analgesia, whereas refeeding-induced analgesia was associated with eating behavior and calorie effect.

Disclosures: **J. Lee:** None. **S. Oh:** None. **Y. Kang:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.01/J40

Topic: D.04. Somatosensation – Touch

Support: MOST106-2314-B182-001
CMRPG5F0092
CMRPG5H0051

Title: Guided neuroplasticity of excitatory neurons in primary sensory cortex: A whisker model

Authors: *Y.-P. CHENG[1], J.-J. HUANG[2], C.-I. YEH[1], Y.-C. PEI[3];
[1]Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Physical Med. and Rehabil., Chang Gung Mem. Hosp., Taoyuan, Taiwan; [3]Chang Gung Mem. Hosp. at Linkou, Taoyuan, Taiwan

Abstract: Neuroplasticity, such as spike timing dependent plasticity, has been investigated primarily based on the change of synaptic weights among few neurons. In this research, we studied the functional plasticity in the topographically organized circuitry of rat barrel cortex (the whisker system) by manipulating activities in a large number of neurons. We paired neural activities induced by stimulating a single whisker [principle whisker (PW) or adjacent whisker (AW)] with those caused by optogenetic stimulation (100 repeats) with different time delays between them. We recorded extracellularly from neurons in the cortical barrel corresponding to the principle whisker, and measured the modulation effect before and after the parings. Direction selectivity for both PW and AW deflections were measured separately by stimulating them in eight directions at 8 Hz before and after pairings. Only one direction was chosen to be paired with light stimulation. Offline sorting was performed to extract single unit signal, and units passed responsive criteria of stimulation would be further analyzed. Our results showed different patterns between PW and AW pairing conditions: pairing induced suppression and the effect was more robust in the AW pairing condition. The suppression was greater when stimulus onset asynchrony (SOA) was 0 ms. Importantly, resting spike activity was altered after pairings, with most of them showing facilitation. We further categorized units with their firing probability induced by whisker stimulation. We found that higher probability units (probability of spikes elicited by stimulation for each trial > 0.5) showed pairing-induced suppression to all whisker directions in both PW and AW pairing conditions, while lower probability units (probability between 0.2 to 0.5) only showed suppression to the paired direction in the AW pairing condition. In summary, neuroplasticity was found by pairing physical stimulation and optogenetic stimulation, which induced synchronous activities among population of neurons. Neural circuitries underlying the plasticity will be further studied.

Disclosures: **Y. Cheng:** None. **J. Huang:** None. **C. Yeh:** None. **Y. Pei:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.02/J41

Topic: D.04. Somatosensation – Touch

Support: NIH Grant HD094588

Title: Structural and functional organization of the lower jaw barrel subfield in rat primary somatosensory cortex

Authors: V. PELLICER MORATA[1], A. L. CURRY[2], L. WANG[1], J. W. TSAO[1], *R. S. WATERS[1,2];
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Abstract: Introduction: The rodent barrel field, associated with the representation of the body surface, is located in layer IV of primary somatosensory (SI) cortex and consists of a number of barrel subfields. Here, we examined the structural organization of the lower jaw barrel subfield (LJBSF) using the metabolic marker, cytochrome oxidase, and related this organization to the functional representation of the lower jaw. Methods: Sprague-Dawley rats (n=19) were anesthetized and the skull was opened to expose the underlying cortical surface. Carbon fiber electrodes were used to map the lower jaw representation in layer IV of SI cortex using mechanical stimulation. Electrode penetration sites were plotted on a surface map of SI cortex and selected penetration sites were identified by making electrolytic lesions. Following mapping, the brain was removed, flattened, and stained with cytochrome oxidase (CO) to produce a barrel map. The surface map was then superimposed on the flattened CO-stained barrel map using lesions to align the two maps. Results: The LJBSF is an ovoid-shaped structure, consisting of individual clusters of cells called barrels that lies on an anteroposterior plane in the rostral barrel field. Within the LJBSF, the posteriorly located barrels, large relative to their anterior counterparts, are associated with the representation of the chin and associated whiskers. The anteriorly-located barrels are associated with the representation of the lower lip. An overlap zone, where neurons respond to input from both chin/whiskers and lower lip, separates the two regions. The visibility of individual barrels in the LJBSF, particularly the most anteriorly-located barrels, made it difficult to precisely relate individual barrel structure to function. Conclusion: We described the detailed organization of the LJBSF and the spatial distribution of the representation of the chin and lower jaw. Our findings are important for understanding the contribution the LJBSF in hand-to-face remapping that follows forelimb deafferentation.

Disclosures: V. Pellicer Morata: None. **A.L. Curry:** None. **L. Wang:** None. **J.W. Tsao:** None. **R.S. Waters:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.03/DP05/J42

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster

Topic: D.04. Somatosensation – Touch

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Title: The sensorimotor strategies and neuronal representations of whisker-based object recognition in mice

Authors: *C. RODGERS[1], R. NOGUEIRA[1], B. C. PIL[1], E. GREEMAN[1], S. FUSI[2], R. M. BRUNO[2];
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Abstract: Humans and other animals can identify objects by active touch - coordinated exploratory motion and tactile sensation. The primary somatosensory cortex (S1) integrates motor, sensory, and cognitive signals and is critical for tactile object recognition. However, the basic principles of computation in the cortex, such as the role of each of its anatomical layers, remain largely enigmatic. We are investigating the sensorimotor strategies mice use to recognize objects and how these strategies are implemented by the flow of information across the cortical layers.

Mice recognize objects by scanning them with their whiskers, just as we do with our fingertips. To study this ability, we trained head-fixed mice to discriminate concave from convex shapes by licking left or right. In order to identify the sensorimotor strategies mice employed, we tracked whiskers in high-speed video and extracted the entire suite of relevant behavioral variables (contact timing, location, bending moment etc.). We then used a linear classifier (logistic regression) to identify the spatiotemporal patterns of contacts that best predicted either the stimulus identity (concave or convex) or the mouse's choice (lick left or right) on each trial. We found that mice successfully processed spatial patterns: contacts by distinct whiskers were correlated with different shapes and drove different choices. In contrast, they did not exploit temporal structure, and indeed integrated information remarkably consistently over the entire duration of the trial.

We next asked how this sensorimotor strategy was implemented by neuronal circuits in S1. We recorded large populations of neurons across all layers and found that they responded to touch, movement, and even complex cognitive signals like reward history. Inhibitory neurons anticipated sensory input by ramping up their firing just before whisker contacts. Excitatory neurons in the superficial layers strongly represented touch whereas the deep layers more strongly encoded task variables like reward. This suggests a model in which superficial neurons integrate touch responses, while deep neurons integrate this touch information in the broader context of the task. Taken together, these studies will reveal the algorithm and neural implementation of object recognition.

Disclosures: C. Rodgers: None. **R. Nogueira:** None. **B.C. Pil:** None. **E. Greeman:** None. **S. Fusi:** None. **R.M. Bruno:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.04/J43

Topic: D.04. Somatosensation – Touch

Support: Fondation pour l'Audition
ERC-CoG DEEPEN
Marie Curie 702726
FRM DEQ20170336761

Title: A new set of higher order stimulus spaces reveals an elaborate slip-stick code in rat barrel cortex

Authors: *E. R. HARRELL[1], M. A. GOLDIN[2], B. BATHELLIER[1], D. E. SHULZ[1];
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Abstract: Computational approaches to sensory processing rely on an understanding of what features are encoded in the brain and how the selectivity for those features is distributed across neuronal populations from the periphery to the cortex. A poor understanding of these features in the whisker system has left computational descriptions of touch in rodents far behind other sensory systems despite being one of the most well-studied sensory pathways in terms of anatomy and development. Using an extended and optimized set of noise-based stimuli delivered through a custom-built multi-whisker stimulator (Jacob et al. 2010), we precisely determined the features that are encoded in deep layer barrel cortex neurons with high-density silicon probe recordings. Through this elaboration of the input space, we identified high-velocity, high-acceleration whisker movements, homologous to slip-sticks observed in natural whisker contacts, as the predominant features encoded by deep layer barrel cortex cells. Our method further demonstrates that neurons can be selective for both the angular position at which the respective 'slip-stick' event occurs and its direction, which links slip-stick coding with earlier studies examining direction tuning. We also show that the recent stimulus history within a 150 ms window plays a crucial role in both feature selectivity and the sparseness of firing to a feature of interest, which is a much longer window than what has been classically studied in the whisker system. This work unifies coding theories derived from behaving rodents with a tightly controlled reverse correlation approach and paves the way forward in unraveling the functional organization of the whisker-barrel system.

Disclosures: E.R. Harrell: None. **M.A. Goldin:** None. **B. Bathellier:** None. **D.E. Shulz:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.05/J44

Topic: D.04. Somatosensation – Touch

Title: Morphological and electrophysiological alterations of layer V pyramidal neurons in the barrel cortex of p53 knock-out mice

Authors: *H. KUANG[1], T. LIU[2];
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Abstract: Recent studies demonstrated that *p53* is involved in aging and neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, possibly due to its role in modulating the neurite outgrowth and axonal regeneration in cultured neurons. However, it is still unknown whether the morphology and function of neurons are altered in *p53* knock-out (KO) mice.
In this study, two age groups of either sex of *p53* KO and wild-type (WT) mice with C57/BL6 genetic background were recruited: juvenile (aged 20-22 days) and young adult (aged 8-12 weeks). Properties of layer V pyramidal cells (L5PC) in the barrel cortex were investigated with whole cell patch-clamp recording and confocal microscope imaging. Morphological classification of L5PC is based on the tuft dendrites and the depth of the soma in the cortex as previously reported: thick tuft cells (TT) and slender tuft cells (ST).
We found that the compositions of morphological types of L5PC in juvenile and young adult *p53* KO mice were not changed compared to those of the WT mice. However, the diameter of the L5PC_ST somata was smaller in juvenile KO mice than WT mice (P = 0.0275), without any changes in tuft height, tuft width and apical dendrite length. Moreover, the normalized pial depth of the L5PC_ST neurons displayed a deeper position in juvenile KO mice compared to that of the WT mice (P =0.0332). Studies on the electrophysiology showed
that the frequency of sEPSCs of L5PC_ST in juvenile *p53* KO mice was slower than WT mice (P = 0.007), without any changes in sEPSCs amplitude and decay time. In adult *p53* KO and WT mice, no significant changes of morphological features were found. However, L5PC_ST also displayed a significantly slower frequency of sEPSCs in adult *p53* KO mice than WT mouse (P = 0.0262). Moreover, longer decay time of sEPSCs was observed in L5PC_TT from adult *p53* KO mice than in WT mice (P = 0.0426). Furthermore, the resistance input of L5PC_TT from adult *p53* KO mice was significantly increased (P = 0.0435), accompanied with a longer half width of action potential in adult *p53* KO mice than control WT mice (P = 0.0252).
In conclusion, these results imply that general knock-out of *p53* may cause alterations in the

morphology and electrophysiology of L5PC in the barrel cortex, which becomes more apparent with the age.

Disclosures: **H. Kuang:** None. **T. Liu:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

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Topic: D.04. Somatosensation – Touch

Support: NSF MRI PHY15326A
NIH NINDS U19 NS107466

Title: Multi-sensory coding by layer 5 pyramidal neurons in mouse vibrissa cortex during active sensation

Authors: *R. LIU[1], M. DESCHÊNES[2], D. KLEINFELD[1];
[1]Dept. of Physics, Univ. of California San Diego, La Jolla, CA; [2]Univ. Laval, Quebec, QC, Canada

Abstract: Layer 5 pyramidal neurons in mammalian cortex are hypothesized to act as a computational hub. One role of this hub is to integrate sensory input and efferent copies of motor control signals during active sensing. In mice, these neurons were shown to contribute to an object localization task based on vibrissa touch (*Ranganathan et al, Nature Neuroscience 2018*). Yet it remains unclear how layer 5 neurons represent specific parameters of the task, with a focus on set-point of the vibrissae, vibrissa touch signals, and concurrent head movement and locomotion. We designed a closed-loop, tactile platform, motivated by work of the Feldman laboratory (Isett et al, 2018 SfN abstract 151.15), that couples locomotion with active sensing via a separate running wheel and motorized tactile roller. The position of the roller is adjusted to lie within the range of exploration of the mouse vibrissae and the rolling speed can be matched or decoupled to the mouse's running speed in real time. Vibrissae position is monitored with high-speed videography. The mice are trained to run for a certain period of time and to simultaneously perform an active vibrissa task that involves contact with the roller in order to obtain a reward. Concurrent with behavior, we use adaptive optics two-photon microscopy to achieve near-diffraction limited imaging of intracellular calcium concentration in layer 5 pyramidal neuron somata and subcellular processes that express jRGECO1a (Liu et al Nature Methods 2019). In preliminary work, we observed robust multi-sensory coding by layer 5 pyramidal neurons for set-point and touch in four logical groups: only set-point, only touch, touch AND set-point, and touch AND NOT set-point. Continuing work further addresses multi-sensory coding by neurons during active sensation.

Disclosures: **R. Liu:** None. **M. Deschênes:** None. **D. Kleinfeld:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

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Topic: D.04. Somatosensation – Touch

Support: Shanghai Municipal Science and Technology Major Project 2018SHZDZX05
General Program of National Natural Science Foundation of China 81771821

Title: Novel paradigms of stimulus evoked fMRI in awake mice

Authors: ***X. CHEN**[1], C. TONG[2], Z. HAN[1], Z. LIANG[1];
[1]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai City, China; [2]Southern Med. Univ., Guangzhou, China

Abstract: At present, animal fMRI studies have increasingly focused on mice [Adamczak et al., 2010, Guilfoyle et al., 2013, Nasrallah et al., 2014, Schlegel et al., 2015] due to the rapid improvement of MRI techniques including ultra-high field and cryogenic surface coil, but most studies used anesthetized mouse preparation for fMRI. It has been reported that the different anesthetics differentially modulated the BOLD activation patterns and time courses [Schroeter et al., 2014, Schlegel et al., 2015]. Furthermore, anesthesia makes the cognitive mapping impractical due to the loss of consciousness in the animal. To overcome the challenge, we established the first awake and behaving mouse fMRI paradigm [Han et al., 2019], and the whole-brain activation patterns during an olfactory Go/No-Go task were obtained. In the current study, we aimed to further optimize the awake mouse fMRI paradigm and characterize spatiotemporal characteristics of the BOLD signal in the awake mouse. High spatial resolution and high temporal resolution fMRI were performed in awake mouse during whisker, auditory and olfactory stimulation. Spatially specific activations were observed at cortical and corresponding thalamic regions for whisker and acoustic stimulation tasks (e.g. activation at contralateral S1BF in unilateral whisker stimulation task). For olfactory stimulation task, robust activation was observed at the olfactory bulb, which is more susceptible to distortion and signal loss. Furthermore, awake mouse specific hemodynamic response function (HRF) was modeled from the high temporal resolution fMRI data, revealing a much faster HRF than human HRF and few regional variations of HRF. Our current work established a whole new set of stimulus-evoked fMRI paradigms in awake mice, with optimized experimental setups, preprocessing strategy and analysis pipeline. The current work would allow further applications of awake mouse fMRI for examining mechanisms of BOLD signals or mapping cognitive functions.

Disclosures: **X. Chen:** None. **C. Tong:** None. **Z. Han:** None. **Z. Liang:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

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Topic: D.04. Somatosensation – Touch

Support: Polish National Science Centre Grant (2015/17/B/NZ4/02016)

Title: Somatostatin interneurons of barrel cortex layer 4 are necessary for plastic change induced by classical conditioning involving whiskers

Authors: *G. DOBRZANSKI, A. LUKOMSKA, R. ZAKRZEWSKA, M. LIGUZ-LECZNAR, M. M. KOSSUT;
Nencki Inst. of Exptl. Biol. PAS, Warsaw, Poland

Abstract: Learning-related plasticity in the cortex was found to be linked to action of disinhibitory circuits of interneurons. In the mice barrel cortex, a Pavlovian conditioning in which stimulation of vibrissae is used as a conditioned stimulus, induces a plastic change of cortical functional representation of vibrissae. This learning-related plastic change can be visualized using functional imaging with [14C]-2-deoxyglucose (2DG) which revealed enlarged representation of vibrissae activated during conditioning. With immunocytochemical investigations we have found that Somatostatin containing GABA-ergic interneurons (SST-INs) were affected by the conditioning. SST-INs are engaged in disinhibition of principal neurons *via* inhibition of Parvalbumin-containing interneurons and they are modulated by Vasoactive Instestinal Peptide containing neurons (VIP-INs).
In the present study we examined with the DREADD technique the involvement of SST-INs and VIP-INs inhibition in the development of the learning-related plastic change. Inhibitory DREADDs were introduced into barrels of row B whiskers in one hemisphere of transgenic SST- or VIP-Cre mice by transduction with Cre-dependent AAV2 vectors. Viral injections into layer IV were made after location of the injection site with optical recording. Electrophysiological recordings from transduced brain slices verified that application of CNO, a DREADD agonist, inhibited spontaneous activity of transduced neurons. Controls for the possible effect of CNO itself in DREADDs-free animals did not reveal its influence on the plastic change examined. The conditioning paradigm consisted of 3 training sessions, CNO was injected before each session and 2DG maps were obtained 24 hours after the last session. In mice with SST-INs blocked during the conditioning the plastic change of whisker representation was absent. The behavioral effect of conditioning, elimination of head movements in response to CS, also did not develop. Inhibition of VIP-INs in layers II-IV during the conditioning did not affect the plastic change. We found that activation of SST-INs is indispensable in the formation of learning-induced plastic change in the barrel cortex.

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Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.09/K2

Topic: D.04. Somatosensation – Touch

Support: BBSRC
Wellcome Trust

Title: Computations in layer 4 barrel cortex during texture discrimination

Authors: *M. PITSIANI[1], C. BUETFERING[1], Z. YANG[1], P. LATHAM[2], M. HAUSSER[1]; [1]Univ. Col. London, London, United Kingdom; [2]Gatsby Computat. Neurosci. Unit, UCL, London, United Kingdom

Abstract: A major question in neuroscience concerns how local cortical networks integrate sensory signals with ongoing activity. Mouse barrel cortex is an area of primary somatosensory cortex that is known to encode whisker-related information. Neurons in layer 4 (L4) receive the main thalamic input encoding low-level whisker kinetics such as speed and curvature. However, the highest proportion of synaptic input to a given L4 neuron originates from neurons in the same layer in barrel cortex. How neurons in L4 integrate the spatiotemporal patterns of various whisker kinetics into distinct tuning curves and the impact of recurrent connectivity in the densely connected L4 are both unclear. Recurrent cortical activity may contribute to signal amplification, increase the signal correlation to smooth responses or even assist the neuronal network to retain working memory. Here, we investigate the interplay of feedforward and recurrent activity of L4 barrel cortex in mice engaged in a texture discrimination task. We are combining the power of experimental and modelling approaches by recording large-scale neural activity using two-photon calcium imaging and analysing neural activity using network modelling. The use of a biologically-inspired and data-driven network model allows us to study the role of recurrent connectivity beyond experimental limitations. Mice successfully performed a two-choice task by discriminating fine differences between textures, the sampling of which results in distinct whisker kinetics. In addition, preliminary results suggest that, in contrast to thalamus, neurons in L4 respond to a combination of whisker kinetics. Activity in strongly modulated L4 neurons is highly informative about the stimulus presented on a trial-by-trial basis. Furthermore, we developed a spiking network model of L4 that is biologically inspired as it

follows probability of connectivity between neurons and spiking properties that are seen in neuronal recording of L4 cells. This model captures the basic neuronal activity during free whisking and will be upgraded to fit L4 activity during texture sampling. By combining large-scale neuronal data from task performing animals with data-driven computational modelling we aim to reveal the computational role of feedforward and local inputs in highly recurrent cortical circuits.

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Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.10/K3

Topic: D.04. Somatosensation – Touch

Title: Developmental connectomics in mouse cerebral cortex

Authors: *A. G. GOUR, K. BOERGENS, P. LASERSTEIN, Y. HUA, M. HELMSTAEDTER; Dept. of Connectomics, Max Planck Inst. For Brain Res., Frankfurt am Main, Germany

Abstract: The development of interneurons in the cerebral cortex has been studied for their migrational origin, time of integration into the cortical plate and electrophysiological properties. While it is known that in adult rodent cortex, the synapses formed by interneurons are highly specific in innervating different subcellular domains, the connectomic development of this target selectivity is however largely unknown. Here, we acquired 3D EM (electron microscopy) datasets from the primary somatosensory cortex in mice at postnatal ages 7, 9, 14 and 28 days to map inhibitory connectomes over ontogenetic development and analyzed the conditional innervation of different subcellular targets (somata, apical dendrites, axon initial segments). We find that there is preferential innervation of different subcellular targets at the earliest circuit stages. Furthermore, we observe that there are distinct time courses for the selective innervation of these targets. The selective innervation of apical dendrites develops first, followed by somata, and finally axon initial segments (AIS). Additionally, our data is consistent with a model of pruning anti-specific synapses during early postnatal development to enhance target selective innervation of somata in adults.
These data provide a first quantification of connectomic specificity over development in the cerebral cortex and open questions to the underlying genetic mechanisms of target identification in the cerebral cortex of mammals

Disclosures: A.G. Gour: None. **K. Boergens:** None. **P. Laserstein:** None. **Y. Hua:** None. **M. Helmstaedter:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.11/K4

Topic: D.04. Somatosensation – Touch

Support: NIMH Intramural IRP Research Program

Title: Functional connectivity of diverse long range inputs to the primary somatosensory cortex

Authors: *S. NASKAR, J. QI, C. R. GERFEN, S. LEE;
NIMH, NIH, Bethesda, MD

Abstract: The mammalian neocortex is parsed into specialized areas that do not operate in isolation but in a concerted manner with other areas through long-range projections, thereby enabling sensory processing and sensorimotor integration. Despite the importance attributed to these long-range communications, it is not clear what rules govern the circuit specificity of such cortico-cortical connections. A previous study has demonstrated that excitatory inputs from the primary motor cortex (M1) strongly recruit vasointestinal peptide (VIP)-expressing GABAergic interneurons (IN) in the primary sensory cortex (S1). The recruitment of VIP INs results in disinhibition of pyramidal neurons by inhibiting somatostatin (SST)-expressing INs. A similar circuit has been observed in other primary sensory areas, including visual and auditory cortices. We asked whether the VIP IN-mediated disinhibition serves a canonical circuit motif for cortico-cortical interactions. Using retrograde viral tracing methods, we first identified the brain areas that project to the supragranular layers of S1. These areas include the primary vibrissa-related motor cortex (vM1), secondary somatosensory cortex (S2), contralateral primary somatosensory cortex (cS1), perirhinal cortex (Prh) and posteromedial thalamic nucleus (POm). Based on this anatomical study, we then investigated the functional connectivity from these major input areas to the different neuronal elements in the superficial layers of S1 using optogenetics and whole-cell patch clamp recordings in *ex vivo* preparations. We found that, in contrast to the VIP IN-mediated disinhibitory circuits from vM1 to S1 projections, long-range projections from sensory cortices including S2 and cS1 strongly recruit fast-spiking parvalbumin (PV)-positive neurons. Regardless of the input areas, SST neurons received the weakest long-range inputs, thus are isolated from long-range connections. Silencing PV INs significantly increased the spike probability of pyramidal neurons upon stimulating S2 axon terminals innervating the superficial layers of S1. In contrast, silencing VIP INs decreased the activity of pyramidal neurons upon stimulating M1 axon terminals. Our results imply that sensory-related feedback information is transmitted to S1 by engaging PV IN-mediated feedforward inhibition, while motor-related feedback information propagates to S1 through VIP IN-mediated disinhibition. Thus, primary

sensory cortex may parse information from diverse feedback projections by means of input area-dependent, preferential recruitment of specific types of GABAergic interneurons.

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Poster

057. Touch: Barrel Cortex Coding

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Topic: D.04. Somatosensation – Touch

Support: NIH Intramural Research Program

Title: Circuit basis for functional heterogeneity of principal neurons in layer II/III of sensory cortex

Authors: *A. R. INACIO, F. PEREIRA, C. R. GERFEN, S. LEE;
NIMH/NIH, Bethesda, MD

Abstract: Principal neurons in layer II/III of sensory cortices exhibit highly asynchronous and heterogeneous activity profiles. Advances in the understanding of layer II/III population activity, both in the context of incoming sensory information and perceptual decision making and in the context of behavioral state, support the existence of patterned activity within heterogeneity. Here, we sought to determine whether long-range and local connectivity rules constrain functional subnetworks of neurons in layer II/III of somatosensory cortex. With two-photon calcium imaging, we first explored spontaneous and externally-driven activity patterns of principal neurons in the barrel cortex of mice. Head-fixed animals were free to run on a wheel, and whisker stimulation was delivered at random times during each recording session. Spontaneous behavior was characterized by bouts of motor activity that consisted predominantly of whisking-alone or whisking-locomotion periods. Quiet periods were coherent with a 3-6 Hz oscillation of local field potential, with motor activity bouts being accompanied by LFP desynchronization, consistent with behavioral-state transitions. Whisking-locomotion periods were particularly salient in the neuronal activity domain, in the form of peaks of synchrony at behaviorally relevant timescales. We found that approximately 30% of all active cells were recruited during whisking-locomotion periods. Of these, approximately 70% showed increased activity, with the remaining 30% showing decreased activity. Importantly, these patterns were highly stable over days and weeks, suggesting that distinct long-range and/or local inputs may dictate the pattern of activity of these neurons. We are currently examining whether functional subnetworks are constrained by specific long-range and local presynaptic ensembles using monosynaptic retrograde tracing methods combined with two-photon calcium imaging. This

study will provide the circuit-based mechanism for functional heterogeneity at the single cell-level.

Disclosures: **A.R. Inacio:** None. **F. Pereira:** None. **C.R. Gerfen:** None. **S. Lee:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.13/K6

Topic: D.04. Somatosensation – Touch

Support: EMBO Long-Term Fellowship
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Wellcome Trust
BBSRC
ERC

Title: Decision coding by layer 2/3 neurons in primary somatosensory cortex

Authors: *C. BUETFERING, M. PITSIANI, J. SMALLRIDGE, M. HAUSSER;
UCL, London, United Kingdom

Abstract: Sensory information enables us to make informed choices that are critical for survival. While primary sensory areas provide information on sensory stimuli, behaviourally-relevant decision-making variables have been shown to be represented in higher-order association cortices. Therefore, sensory coding and decision-making are typically studied under the assumption of anatomical separation. Neurons in the superficial layers of the whisker region of primary somatosensory cortex (S1), barrel cortex, not only receive somatotopically mapped bottom-up inputs from the thalamorecipient layer 4 but also lateral projections from neighbouring barrels and top-down projections from higher cortical areas. Therefore, layer 2/3 (L2/3) neurons in barrel cortex are a prime candidate for providing an intersection of sensory processing and decision-making in complex behavioural tasks. Previous work using electrophysiological recordings in monkeys, rats and mice has not found conclusive choice activity in S1 but was limited to low number of neurons. Studies using two-photon calcium imaging found that some behavioural aspects modulate activity in L2/3 barrel cortex neurons. It is unclear, however, whether the signal difference across trial types in those studies reflects choice-related signals or a modulation of activity by action-related variables such as motivation, movement preparation etc. Here, we used two-photon calcium imaging of neurons in L2/3 mouse barrel cortex during a cued texture discrimination task with two lickports to determine whether these neurons can code for behaviourally-relevant decision variables. We found neurons carrying information about the stimulus irrespective of the behavioural outcome (‘stimulus neurons’) as

well as neurons whose activity carried information about the choice to be made ('decision neurons'). Choice-related activity in decision neurons is not driven by signals related to motor output, but instead follows stimulus presentation. Furthermore, ambiguous population coding of decision neurons predicts miss trials and an improvement in categorical coding in decision neurons coincides with learning the stimulus-choice association. Our identification of neurons encoding stimulus and behaviourally-relevant decision signals within the same circuit suggests a direct involvement of L2/3 S1 in the decision-making process.

Disclosures: **C. Buetfering:** None. **M. Pitsiani:** None. **J. Smallridge:** None. **M. Hausser:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

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Program #/Poster #: 057.14/K7

Topic: D.04. Somatosensation – Touch

Support: ERC AdG 695709
Wellcome Trust PRF 201225
BBSRC BB/N009835

Title: Two-photon all-optical interrogation of layer 2/3 mouse barrel cortex during a sensory discrimination task

Authors: *O. M. GAULD[1], A. M. PACKER[1,2], L. E. RUSSELL[1], M. HAUSSER[1];
[1]Univ. Col. London (UCL), London, United Kingdom; [2]Univ. of Oxford, Oxford, United Kingdom

Abstract: To identify links between neural activity and behaviour it is important to measure and manipulate neural circuits with cellular resolution in awake behaving animals. Recent developments in simultaneous 'all-optical' two-photon calcium imaging and optogenetic photostimulation are ideally suited for this type of investigation (Packer *et al*, 2015). The coupling of a programmable spatial light modulator (SLM) into the photostimulation light path enables neurons to be selectively manipulated based on their functional identity, which has not been possible using conventional optogenetic strategies. We combined this novel technology with a two-alternative forced choice (2AFC) behavioural task to investigate sensory encoding and perceptual decision-making in head-fixed mice. Mice were trained in a psychometric task to discriminate bilateral whisker input and report their decisions with a left/right licking response after a short delay period. Once trained, we performed volumetric two-photon calcium imaging of GCaMP6s in the corresponding barrel column in primary somatosensory cortex during behaviour. Layer 2/3 neurons showed responses that were modulated by contralateral and

ipsilateral whisker input and predictive of upcoming behavioural choice. Using co-expression of GCaMP6s and a somatically restricted opsin (C1V1-KV2.1), we are photostimulating stimulus and choice-informative neurons specifically during behaviour to explore the mechanisms by which sensory information is encoded in cortical circuits, and how this information informs behaviour.

Disclosures: O.M. Gauld: None. **A.M. Packer:** None. **L.E. Russell:** None. **M. Hausser:** None.

Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

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Program #/Poster #: 057.15/K8

Topic: D.04. Somatosensation – Touch

Title: Inter-columnar interactions in layers 2-4 of barrel cortex in behaving mice

Authors: *B. VOELCKER[1], R. PANCHOLI[2], S. P. PERON[2];
[1]Ctr. for Neural Sci., NYU, New York, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The majority of connections in primary sensory cortex are local, yet the impact of local synaptic interactions on neural responses to sensory stimuli remains poorly understood. Two canonical network motifs that have been implicated in local processing are lateral inhibition and feed-forward excitation. Given two spatially intermingled populations preferring distinct sensory stimuli, lateral inhibition can manifest as mutual suppression between these populations, whereas feed-forward excitation can manifest in the form of higher-order neurons responding to both stimulus classes. We report evidence consistent with both lateral inhibition and feed-forward excitation in primary somatosensory cortex (vS1; 'barrel' cortex) of mice performing a vibrissal object localization task. By trimming mice to two adjacent whiskers, we examine the interactions between the networks of neurons responding to each whisker. Using 2-photon calcium imaging in transgenic mice expressing GCaMP6s exclusively in excitatory neurons (Ai162 X Slc17a7-Cre), we monitor neural activity from thousands of neurons in layer (L) 4 and L2/3 over a field of view spanning the 2 barrel columns corresponding to the intact whiskers. In L2/3, we find cells consistently responding to touches from only one of the two spared whiskers, as well as cells responding to touches to either one of the intact whiskers. Many single-whisker cells showed a marked decrease in response to touch of their preferred whisker when the touch was preceded by contact of the adjacent, consistent with lateral inhibition between the two single-whisker populations. L4 cells were more likely to respond exclusively to stimulation of the whisker corresponding to their barrel, and showed minimal suppression by preceding adjacent whisker touches, suggesting that the suppression in L2/3 may be due to local circuitry.

Multi-whisker neurons were also far less frequent in L4, and even within L2/3, they increased in frequency more superficially. Given that connections between L2/3 neurons are common across barrel columns, this data is consistent with multi-whisker neurons pooling the responses of single-whisker neurons. Together, our results suggest that local circuitry in L2/3 of vS1 transforms vibrissal touch responses. Future experiments will directly test whether lateral inhibition and feed-forward excitation emerge as a consequence of local interactions.

Disclosures: B. Voelcker: None. **S.P. Peron:** None. **R. Pancholi:** None.

Poster

057. Touch: Barrel Cortex Coding

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Topic: D.04. Somatosensation – Touch

Support: Richard and Susan Smith Family Foundation
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F31NS111896

Title: A molecular and anatomical characterization of neuronal cell types in layer 2/3 of primary somatosensory cortex

Authors: *C. J. CONDYLIS[1], K. BISTRONG[2], Z. YAO[3], K. SMITH[3], T. NGUYEN[3], B. TASIC[3], H. ZENG[3], J. L. CHEN[2];
[1]Biomed. Engin., [2]Biol., Boston Univ., Boston, MA; [3]Allen Inst. For Brain Sci., Seattle, WA

Abstract: Individual neurons in the mammalian neocortex can potentially be defined by their anatomical, molecular, and functional properties. To understand what defines a cell type and its role in a circuit, we set out to link anatomical and molecular properties of neurons in the mouse primary somatosensory cortex (S1). To determine if the molecular properties of layer 2/3 (L2/3) in S1 were similar to that of other cortical areas, single cell RNA sequencing in L2/3 of S1 was compared to that of L2/3 of primary visual cortex (V1) and anterior lateral motor cortex (ALM). Clustering analysis showed that S1 inhibitory neurons form clusters that include both V1 and ALM inhibitory neurons, reinforcing the idea that inhibitory neuron cell types are similar across the cortex. Excitatory neurons in S1 formed three molecular clusters that were more similar to neurons in V1 than ALM. We then asked if each of these transcriptionally-defined excitatory cell types in S1 shared anatomical features by examining their long-range axonal projection targets. L2/3 excitatory neurons in S1 were labelled by a fluorescently-tagged retrograde tracer, cholera toxin subunit-b (CTB), to identify neurons that projected to secondary somatosensory cortex

(S2), primary motor cortex (M1), contralateral S1 (cS1), or striatum (Str). Fluorescence *in situ* hybridization (FISH) targeting transcripts informative of cell-type cluster was subsequently performed on the CTB-labelled cells. We found graded differences between projection populations and cell type marker. Neurons projecting to S2 were underrepresented in cell types defined by *penk* and *ptgs2* expression. Neurons projecting to M1 were overrepresented in a cell type defined by *s100a6* expression. However, no one-to-one correspondence between molecular clusters and anatomical projection target were observed. These findings suggest that L2/3 S1 neurons represent a heterogeneous population with highly overlapping anatomical and molecular properties, spurring future work to understand how these properties relate neuronal function.

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Poster

057. Touch: Barrel Cortex Coding

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 057.17/K10

Topic: D.04. Somatosensation – Touch

Support: HHMI Gilliam Fellowship

Title: Context dependent sensorimotor processing during active sensation

Authors: *G. I. TELIAN[1], J. BROWN[1], H. ADESNIK[2];
[1]Univ. of California Berkeley, Berkeley, CA; [2]Univ. of California, Berkeley, Berkeley, CA

Abstract: Mice actively adapt their whisking strategy to explore their environment. Vibrissae somatosensory cortex (vS1) is thought to convey sensory information to vibrissae motor cortex (vM1), via direct cortico-cortico connections, in order to rapidly update the whisker motor program. However, this hypothesis has not been explicitly tested. Here we compare vM1's role in sensory-motor processing between untrained mice, and animals performing a whisker-dependent discrimination task. In both contexts, animals were head-fixed and free to palpate the tactile stimulus. Using high-density extracellular recordings, optogenetic silencing, and high speed videography we probe the sensory representation of tactile stimuli in vM1 and ask whether vM1 activity is necessary for adaptive whisking strategies.We find that vM1 represents tactile stimuli independent of vS1, putatively relying on its direct thalamocortical input. In untrained mice, vM1 silencing had no detectable impact on whisking kinematics, but had a dramatic impact when mice were engaged in the tactile discrimination task. vM1 silencing also dramatically impaired performance on the task which is a potential consequence of both impaired adaptive whisking and the elimination of refined sensory representations in vS1 from the direct vM1 feedback connections. We conclude that vM1 can encode the tactile environment

independent of S1, and its control over whisker movements is context dependent, being specifically engaged during demanding sensory tasks.

Disclosures: **G.I. Telian:** None. **J. Brown:** None. **H. Adesnik:** None.

Poster

057. Touch: Barrel Cortex Coding

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Title: Circuits underlying tactile object detection

Authors: *Y. K. HONG, B. C. PIL, E. A. GREEMAN, R. M. BRUNO;
Columbia Univ., New York, NY

Abstract: For many cortical areas, the effect of transient vs. chronic inactivation can yield dramatically different effects on sensory-guided behavior. For a whisker-mediated object detection task, transient inactivation of barrel cortex (S1) can impair sensory detection but this effect is only temporary; lesioning S1 impairs behavior on the first day after lesion but mice fully recover after a single session of re-exposure to the task, bypassing any requirement for S1 in sensory detection. What is the mechanism by which transient, but not chronic, inactivation disrupts detection behavior? Cortical and subcortical areas may encode redundant sensory information and either may be sufficient for sensory detection. An alternative hypothesis is that sudden inactivation of S1 may impair behavior, not because the sensory information encoded in S1 is important, but because this sudden manipulation is disruptive to downstream areas that are essential for the sensory detection behavior. An essential step in understanding sensory behavior is to identify these alternate pathways that can mediate detection, even the absence of S1. S1 has over a dozen different subcortical targets that may be affected by the inactivation of all S1 output neurons. In order to narrow down the specific downstream targets essential to sensory detection, we selectively inactivated different subsets of cortical neurons that project to different target regions, and determined the effect of inactivation on detection behavior. In particular, we find that optogenetically silencing intratelencephalic neurons in S1 yielded no behavioral deficit,

while inactivation of pyramidal tract neurons impaired behavior, suggesting in important role for subcortical projections in mediating tactile behavior. Ongoing work is aimed at identifying the precise subcortical circuitry that mediates detection behavior in the absence of S1 activity.

Disclosures: Y.K. Hong: None. **B.C. Pil:** None. **E.A. Greeman:** None. **R.M. Bruno:** None.

Poster

057. Touch: Barrel Cortex Coding

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Topic: D.04. Somatosensation – Touch

Support: NIH R01 NS045130
Fulbright Graduate Study Award
Carney Institute for Brain Science Graduate Research Award

Title: S1 neurons with the highest spontaneous firing rate are the most informative of success in a detection task

Authors: *H. SHIN[1], C. I. MOORE[2];
[1]Brown University, Neurosci., Providence, RI; [2]Neurosci., Brown Univ., Providence, RI

Abstract: Perceptual decision making transforms sensory stimulation into behavior. For somatosensory stimuli, primary somatosensory neocortex (SI) is a key node in this transformation. Detailed analysis of ongoing neural activity, including both the rate and temporal pattern of spiking, is important for deciphering how sensory relay is achieved. Here, we approached this question by employing tetrode recordings in SI barrel cortex from mice performing a precisely controlled vibrissae deflection detection task. We find that neurometric performance (N) is worse than the mouse's psychometric performance (P) for the vast majority of SI regular-spiking units (RS, putative excitatory neurons; N=273/278 N<P). This result holds even when considering only sensory responsive RS (N=53/56 N<P), contrasting prior reports from higher visual area in monkey visual cortex (Newsome, Britten & Movshon, 1989). Interestingly, fast-spiking units (FS, putative inhibitory neurons) contained a higher proportion of units that had better neurometric than psychometric performance (N=27/188 FS, N=23/69 sFS N≥P). These results suggest that information in SI is likely not integrated in accordance to the "population pooling model" developed by Newsome and colleagues (1989, 1992). Instead, there is an apparent inequality in how informative (or predictive) an SI neuron is about an upcoming perceptual decision. We find that the mutual information contained in a neuron's firing rate about stimulus amplitude and behavior, as well as the area under the receiver-operating characteristic curve discriminating stimulus amplitude-matched hits and misses (detect probability DP), correlated with spontaneous firing rate levels. In other words, higher baseline

firing rate correlated with the neuron's ability to predict successful detection, for both RS and FS. This correlation has implications for how information may be integrated within SI.

Disclosures: H. Shin: None. **C.I. Moore:** None.

Poster

057. Touch: Barrel Cortex Coding

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Topic: D.04. Somatosensation – Touch

Support: NSF GRFP to JMP
NIH F32 NS096819
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NIH R01 NS094659

Title: Primary somatosensory cortex is essential for texture discrimination but not object detection in mice

Authors: *J. PARK[1], C. RODGERS[1], Y. HONG[2], J. DAHAN[1], R. M. BRUNO[3];
[2]Dept. of Neurosci., [3]Neurosci., [1]Columbia Univ., New York, NY

Abstract: The sense of touch is a fundamental part of our sensory experience, yet our understanding of the underlying neural circuitry is limited. For example, the primary somatosensory cortex (S1) is generally known to be involved in tactile processing. Yet, surprising results from our lab has recently shown that S1 is completely dispensable for a whisker-based go/no-go object detection task in mice. We therefore asked whether finer discrimination of tactile stimuli requires the greater computational power of the cortex. In order to test this, we developed a two-alternative forced choice (2AFC) paradigm in which head-fixed mice are trained to either (1) detect objects or (2) discriminate between two textures using only their whiskers. Mice readily learn both tasks at various distances (passive and active) using all or even a single whisker. We then lesioned S1 to test whether the cortex is required for each task. Consistent with previous results from the go/no-go paradigm, animal performance for object detection using the 2AFC paradigm rapidly recovers following S1 lesion. However, lesioning S1 reduces texture discrimination performance to chance, and mice are unable to recover even after many days of testing. Thus, S1 is essential for assessment of a tactile quality (texture discrimination), and task design (go/no-go versus 2AFC) is not a critical determinant of cortical dependence.

Disclosures: J. Park: None. **C. Rodgers:** None. **Y. Hong:** None. **J. Dahan:** None. **R.M. Bruno:** None.

Poster

057. Touch: Barrel Cortex Coding

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Topic: D.04. Somatosensation – Touch

Support: SFARI grant 479737

Title: Nicotinic acetylcholine receptor inhibition in primary somatosensory cortex improves performance during a sensory-guided decision-making task

Authors: *M. F. OGINSKY[1], S. E. KWON[2];
[1]MCDB, Univ. of Michigan, Ann Arbor, MI; [2]MCDB, Univ. of Michigan-Ann Arbor, Ann Arbor, MI

Abstract: Proper integration of neuromodulatory input with cortical networks is critical for correct decision-making in sensory-guided tasks. Neuromodulators such as acetylcholine (Ach) are released in cortical areas during behaviorally relevant sensory cues. Inducing Ach release during a sensory cue in the medial prefrontal cortex enhances *correct* behavioral responses but can also enhance *incorrect* responses when there is no cue. This suggests that cholinergic dysfunction in cortical areas may contribute to making incorrect decisions. However, it is unclear whether and how Ach affects *sensory* cortical function in tasks that involve sensory-guided decision making. To test this, we infused Ach receptor antagonists into the primary vibrissal somatosensory cortex (vS1), a critical area for sensory-guided decision-making, prior to the task using a Go/NoGo paradigm. During this Go/NoGo task a mouse licks for a water reward when its whiskers are stimulated (Go trials) but should withhold licking without whisker stimulation (NoGo trials). We found that blocking nicotinic Ach receptors (nAChRs) in vS1 improved performance by reducing incorrect behavioral responses. Specifically, the fraction of correct trials was enhanced and the false alarm rate was decreased without any changes to the hit rate. Next, we analyzed the licking pattern and found that blocking nAChRs decreased the total number of licks during NoGo trials but not during Go trials. The number of licks was reduced during the pre-whisker stimulation window across all trials. Muscarinic antagonists had no effect on performance. These data suggest that nAchRs in vS1 may be enhancing impulsive behaviors in the absence of the conditioned stimulus, which is consistent with nAChR activation effects found in medial prefrontal cortex. We are combining optogenetics with *in vivo* two photon calcium imaging of vS1 to elucidate the mechanisms involved with ACh regulation of sensory-guided decision-making.

Disclosures: M.F. Oginsky: None. **S.E. Kwon:** None.

Poster

057. Touch: Barrel Cortex Coding

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Topic: D.04. Somatosensation – Touch

Support: UZH Forschungskredit Candoc

Title: Specific $GABA_A$ receptor subtypes influence sensory input processing through gephyrin scaffold dynamics

Authors: *Y.-C. TSAI[1], M. HLEIHIL[1], J. STOBART[2], K. FERRARI[1], B. WEBER[1], S. TYAGARAJAN[1];
[1]Inst. of Pharmacol. and Toxicology, UZH, Zurich, Switzerland; [2]Col. of Pharm., Winnipeg, MB, Canada

Abstract: In the somatosensory cortex, GABAergic inhibition controls the cortical circuit by shaping the sparse coding property of the incoming sensory information. However, the mechanisms involved in this sensory encoding are still unclear. In this *in vivo* study, we examined the contribution of postsynaptic GABAergic components, namely $GABA_AR$ and scaffolding protein gephyrin for sensory encoding. In the mouse barrel cortex, we studied the changes in activity of pyramidal neurons by expressing a genetically encoded Ca^{2+} indicator RCaMP1.07 and populations of layer2/3 (L2/3) pyramidal neurons were imaged in spontaneous or whisker stimulation trials. As a first aim, we dissected the specific roles of alpha1- and alpha2-containing $GABA_ARs$ in sensory information processing. To achieve this, transgenic *Gabra1* KO and *Gabra2* KO mice were imaged under two-photon (2P) microscope. Our results showed that alpha1-containing receptors are important for controlling the population (number of neurons) responding to whisker stimulation, while alpha2-containing $GABA_ARs$ contribute to the neuronal response fidelity during whisker stimulation. In order to understand the underlying mechanism, we focused on the main inhibitory synapse scaffolding protein gephyrin. By using a combination of gephyrin mutations that organize the scaffold differentially, and with a viral delivery approach, we co-expressed RCaMP and eGFP- gephyrin in pyramidal neurons in the barrel cortex. We followed the neuronal populations over several imaging sessions and evaluated change in Ca^{2+} transients within these neurons before and after gephyrin mutant expression. We present data that places gephyrin as a central signalling hub facilitating neuronal excitability, as disruption of gephyrin scaffold or its stabilization increases and decreases the Ca^{2+} transients in pyramidal neurons. Interestingly, gephyrin post-translational modification(s) shape its preference for alpha1 or alpha2-containing $GABA_ARs$ during sensory information processing.

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Poster

057. Touch: Barrel Cortex Coding

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Topic: D.04. Somatosensation – Touch

Support: 479737

Title: Cholinergic modulation of cortical-cortical feedback pathways

Authors: *C.-W. CHANG, S. KWON;
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Abstract: Primary sensory cortex receives long-range inputs from higher cortical areas and neuromodulatory centers. The intracortical feedback connections are found ubiquitously in the mammalian neocortex and modulate sensory processing, attention, learning and sensorimotor integration. Cholinergic projections to sensory cortex also play diverse modulatory roles in attention, reinforcement learning and context-dependent sensory processing. Despite a wide array of functions served by intracortical and cholinergic inputs, whether and how they interact within the cortical microcircuit remains elusive.

To address this issue, we first determined synaptic input-output relationships between an intracortical feedback input and local neurons, and asked whether specific synapses are under cholinergic modulation. Primary somatosensory cortex (S1) in rodents receives a major glutamatergic feedback from secondary somatosensory cortex (S2) and a cholinergic input from basal forebrain. On acute slices prepared from 6-10 week-old mouse with channelrhodopsin-2 expression in S2 area, we performed sequential paired voltage-clamp whole-cell recordings from genetically identified GABAergic interneuron sub-types and neighboring excitatory neurons in S1 area. By optogenetically stimulating S2->S1 projection to evoke synapse-specific evoked excitatory postsynaptic current (eEPSC), we were able to determine relative synaptic weights of S2->S1 projection to each interneuron subtype by comparing the response amplitude between the paired inhibitory neuron subtype and excitatory neuron. We found that parvalbumin (PV)-expressing interneurons and somatostatin (SST)-expressing interneurons exhibited significantly stronger optogenetic eEPSC than their excitatory neuron partners, while vasointestinal peptide (VIP)-expressing interneurons displayed comparable eEPSC (N = 10, 12, 9 pairs for PV, SST, VIP neurons). This result suggests PV and SST interneurons are the main circuit component that mediates S2->S1 modulation.

Further, we measured the eEPSC magnitude and paired-pulse ratio in presence or absence of a cholinergic agonist (carbachol) to test whether specific synapses were modulated. We found that the cholinergic agonist modulated S2 synaptic input to PV and SST neurons. These findings suggest that intracortical feedback recruits specific interneuron sub-types in a target area and that

these synaptic partners represent a substrate of cholinergic modulation. Our results reveal critical nodes of interaction between intracortical and cholinergic modulations in the mammalian neocortex.

Disclosures: C. Chang: None. **S. Kwon:** None.

Poster

057. Touch: Barrel Cortex Coding

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Boston University Center for Systems Neuroscience Post-Doctoral Fellowship

Title: Context dependent sensory processing across primary and secondary somatosensory cortex during a tactile working memory task

Authors: C. CONDYLIS[1], E. LOWET[2], J. NI[2], K. BISTRONG[2], T. OUELLETTE[2], N. JOSEPHS[3], E. D. KOLACZYK[3], ***J. L. CHEN**[2,1];
[1]Biomed. Engin., [2]Biol., [3]Mathematics and Statistics, Boston Univ., Boston, MA

Abstract: A critical feature of sensory perception is the ability to extract meaning by comparing stimulus information across time. To investigate the role of primary (S1) and secondary (S2) somatosensory cortex during this process, we developed a head-fixed, whisker-based version of a delayed non-match-to-sample task. Using a recently developed multi-area two-photon microscope, we performed simultaneous calcium imaging of neuronal populations across layer 2/3 of S1 and S2 in expert mice performing the task. Additional retrograde viruses were delivered in S1 and S2 to anatomically identify feedforward and feedback projection neurons between the two areas. Activity encoding current stimuli, past stimuli, along with categorical "match" and "non-match" responses reflecting the comparison between past and stimuli were observed across both areas. Feedforward neurons preferentially signaled present information while feedback neurons preferentially signaled past information. Optogenetic inactivation experiments using VGAT-ChR2 mice support the involvement of S1 in processing present stimulus information and S2 in processing past stimulus information. Analysis of error trials and passive conditions suggest that past and categorical information is modulated by behavioral state

but more persistently encoded in S2. Network analysis indicate that match and non-match neurons in S2 exhibit prominent functional coupling between each other and across similar neurons in S1. These results such that context dependent sensory processing emerges through an inter-areal network involving S1 and S2.

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Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.01/K18

Topic: D.05. Olfaction and Taste

Support: NIH Grant R01 GM130136
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Title: Parallel plastic sensory pathways are integrated to generate state-dependence in *C. elegans* chemosensory behavior

Authors: J. LUO, *D. S. PORTMAN;
Univ. of Rochester, Rochester, NY

Abstract: Sex differences in innate behavior provide an opportunity to understand how biological sex modifies the development and function of neural circuits. In the nematode *C. elegans*, the two sexes (males and self-fertile hermaphrodites) differ in their innate behavioral responses to the ascaroside-family sex pheromone ascr#3. Adult males are strongly attracted to this cue, providing a mechanism for locating mates, while adult hermaphrodites are weakly repelled, perhaps responding to ascr#3 as a population-density cue. By examining mutants with altered neuropeptide signaling, we found that PDFR-1, a receptor for the PDF family of neuropeptides, can modulate these behavioral responses. *pdfr-1* mutant hermaphrodites exhibit strongly enhanced repulsion by ascr#3; in contrast, the response to ascr#3 is largely unaffected in *pdfr-1* males. Two *pdfr-1* ligands, *pdf-1* and *pdf-2*, act partially redundantly to activate *pdfr-1* signaling. We found that the strong repulsion in *pdfr-1* hermaphrodites requires the ASI chemosensory neuron pair as well as the BMP-family factor *daf-7*, which is produced exclusively by ASI in hermaphrodites. Previous work from our lab has shown that a different pair of sensory neurons, ADF, mediates ascr#3 attraction in males. ADF-ablated males, like wild-type hermaphrodites, are weakly repelled by ascr#3, suggesting that ADF signaling might override a sex-shared repulsive drive. Consistent with this, we found that ADF-ablated *pdfr-1* mutant males exhibit strong ascr#3 repulsion, resembling *pdfr-1* hermaphrodites. Moreover, simultaneous ablation of ADF and ASI in *pdfr-1* males eliminated all behavioral responses to

ascr#3. Together, these results suggest pheromone response behavior results from the integration of a repulsive pathway, mediated by ASI and modulated by *pdfr-1*, with an attractive pathway, mediated by ADF and modulated by biological sex. We are currently working to identify the site of *pdfr-1* action in the pheromone circuit and to determine whether ASI can detect ascr#3 directly. Understanding how these pathways are integrated and how their modulation enables behavioral flexibility should provide more general insight into state-dependent neural circuit function and its underlying mechanisms.

Disclosures: J. Luo: None. **D.S. Portman:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.02/K19

Topic: D.05. Olfaction and Taste

Support: NIH Grant NS087544

Title: Sleep alters the physical architecture of sensory synapses in C.elegans

Authors: *F. FARAH[1], A. BOKKA[1], E. CHANG[1], A. VARSHNEY[1], J. LI[1], C. ECHEVERRIA[1], A. BARADWAJ[1], I. SIMAN-TOV[1], D. COTO VILLA[1], K. ANDERSEN[1], S. ALLADIN[1], F. MUÑOZ-LOBATO[2], K. BENEDETTI[2], S. NORDQUIST[2], N. L'ETOILE[2], M. VANHOVEN[1];
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Abstract: Sleep is critical for the consolidation of memory. However, little is known about the effects of sleep on specific neuronal connections, though the modulation of these connections is thought to underlie learning and memory. We have focused our studies on the AWC olfactory circuit in *C. elegans*, which mediates attraction to several odorants, including butanone. Using the fluorescent split GFP-based trans-synaptic marker Neuroligin 1 GFP Reconstitution Across Synaptic Partners (NLG-1 GRASP), we have identified specific synaptic connections in the AWC olfactory circuit that are altered in a sleep-dependent manner after training with butanone. Specifically, we find that in animals that are trained three times with butanone and without food, then allowed to sleep on food for two hours, and recover on food for 14 more hours, synapses between AWC olfactory neurons and AIY interneurons are reduced in comparison with animals trained with a control buffer, but otherwise treated identically. The reduction in AWC-AIY synapses is not observed in animals that are deprived of sleep for the two hours immediately following butanone or buffer training by placing them on a plate without food, and then allowed to recover on food for 14 hours. We are currently focusing on determining the timeline of the synaptic changes using time course experiments. By discovering how memory is consolidated at

the level of specific neuronal connections, we will set the stage to understand the basic cellular and molecular mechanisms of sleep, and develop more effective treatments for diseases in which memory consolidation is altered, such as Alzheimer's disease and dementia.

Disclosures: F. Farah: None. **A. Bokka:** None. **E. Chang:** None. **A. Varshney:** None. **J. Li:** None. **C. Echeverria:** None. **A. Baradwaj:** None. **I. Siman-Tov:** None. **D. Coto Villa:** None. **K. Andersen:** None. **S. Alladin:** None. **F. Muñoz-Lobato:** None. **K. Benedetti:** None. **S. Nordquist:** None. **N. L'Etoile:** None. **M. VanHoven:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.03/K20

Topic: D.05. Olfaction and Taste

Support: NIH Grant DC009839
NIH Grant DC015525

Title: Odor processing by distinct classes of principal neurons in piriform cortex

Authors: *S. NAGAPPAN, B.-X. HAN, F. WANG, K. M. FRANKS;
Neurobio., Duke Univ., Durham, NC

Abstract: Odors activate distributed populations of principal neurons in piriform cortex (PCx). Principal neurons in PCx are largely concentrated within Layer II (LII), which subdivides into a superficial and a deep layer, each containing a morphologically and electrically distinct class of excitatory neurons. Semilunar cells (SLs) in superficial LII are thought to receive afferent inputs predominantly from the olfactory bulb (OB) while superficial pyramidal cells (SPs) in deep LII are thought to receive both OB and intracortical inputs. This difference in connectivity between SLs and SPs, determined in acute mouse brain slices, suggests a two-stage model for odor processing in PCx, where SLs pre-process afferent OB inputs, and then transmit this information to SPs. The model predicts that SLs should be more narrowly tuned than the recurrently connected SPs and SL odor responses should precede SP odor responses.
To determine the roles of SLs and SPs in odor processing *in vivo*, we generated a NetrinG1-Cre mouse line that selectively expresses Cre recombinase in SLs. We then used cre-dependent AAVs to express Archaeorhodopsin-T (Arch) in SLs and recorded both light- and odor-evoked spiking in large populations of LII neurons in awake, head-fixed mice. Cells with rapid and robust light-induced suppression were determined to be Arch+ SLs, while Arch- cells were presumptive SPs. Interestingly, we found that SLs were in fact more broadly tuned than SPs and odor response latencies were similar between SLs and SPs.
To test the prediction that SPs are driven by SL input, we compared SP odor responses under

control conditions and when SLs were selectively silenced. SL suppression decreased spontaneous firing in SPs, indicating that SPs are, at least, partially driven by SLs. Surprisingly, however, not only did SL suppression not abolish odor responses in SPs, but SP population responses were largely unaffected. This suggests that odor processing by SLs is not required for the activation of SPs, as proposed by the sequential model. Additionally, because SLs and SPs project odor information to distinct downstream regions, our data suggest that SLs and SPs may represent parallel and largely independent odor processing streams.

Disclosures: **S. Nagappan:** None. **B. Han:** None. **F. Wang:** None. **K.M. Franks:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.04/K21

Topic: D.05. Olfaction and Taste

Support: JSPS KAKENHI Grant Number JP 19J20733
JSPS KAKENHI Grant Number JP 18J21358
JSPS KAKENHI Grant Number JP 18H05088
JSPS KAKENHI Grant Number JP 16H02061
JSPS KAKENHI Grant Number JP 18K11485
JSPS KAKENHI Grant Number JP 16K14557
The cosmetology Research Foundation

Title: Association of cue odor signals with predicted behavioral scene signals in piriform cortex neurons

Authors: *Y. TANISUMI[1], K. SHIOTANI[1], K. MIURA[2], J. HIROKAWA[1], Y. SAKURAI[1], K. MORI[3], H. MANABE[1];
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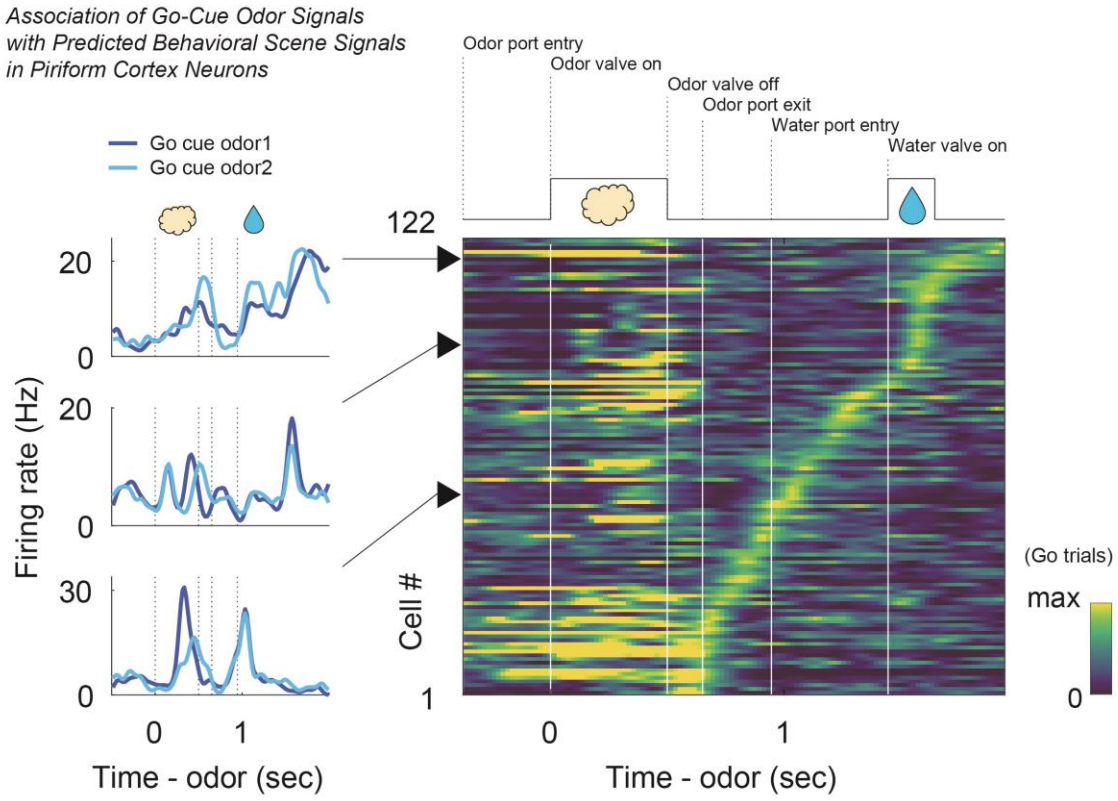
Abstract: Neurons in the piriform cortex receive not only olfactory sensory signals but also top-down cognitive signals originated in higher order regions. However, it still remains a mystery as to how these top-down cognitive signals are expressed in the piriform cortex and associated with olfactory sensory signals. Here, we recorded neural ensemble activity in the anterior piriform cortex (aPC) of freely moving rats performing a go/no-go association task using four cue odors and its reversal. In each session, two odors induced the rat to enter the reward port (go trial), and another two induced the rat to stay near the odor port to wait for the next trial (no-go trial). During odor checking scene at the odor port, many aPC neurons responded to a specific

combination of cue odors. In addition, a majority of them showed increased firings during a specific scene of subsequent behaviors that were predicted by the cue odors (i.e., moving to the reward port scene, reward waiting scene, drinking scene, and waiting scene on no-go trials). For example, a subset of aPC cells that responded to go-cue odors showed increased firings during the subsequent drinking scene, suggesting the association between the go-cue odor recognition during the odor checking scene and the drinking of reward water in the subsequent drinking scene. Furthermore, we recorded the change in the firing pattern of these aPC neurons during the reversal learning after the change of odor-reward association rule. Interestingly, most of the neurons which responded to the cue odors at the odor checking scene and to a specific subsequent behavioral scene maintained their scene-selective firings even after the reversal learning, whereas the firing pattern during odor checking scene exhibited various changes in the course of the reversal learning. Based on these results, we speculate that aPC neurons play an important role in the association between cue-odor representations and top-down cognitive signals that occur during odor-guided learned behaviors.



Disclosures: **Y. Tanisumi:** None. **K. Shiotani:** None. **K. Miura:** None. **J. Hirokawa:** None. **Y. Sakurai:** None. **K. Mori:** None. **H. Manabe:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.05/K22

Topic: D.05. Olfaction and Taste

Support: NIH/NIDCD Grant R01DC014443

Title: Local field potential dynamics during odor-directed attention

Authors: *H. L. CANSLER, E. E. IN 'T ZANDT, D. W. WESSON;
Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Sensory representations in the brain are modulated by internal states and cognitive factors, like attention. In the olfactory system, the olfactory tubercle (OT) exhibits attentional modulation of odor-evoked activity in a manner that increases signal-to-noise, much like what occurs in other sensory cortices. The circuit mechanisms that underlie attentional modulation of sensory processing are well-studied in other sensory systems, and involve top-down inputs from the prefrontal cortex to the sensory thalamus. In the olfactory system, wherein sensory information is conveyed directly to the olfactory cortex without thalamic processing, the circuit mechanisms underlying attentional modulation of sensory processing are unknown. To identify candidate circuits involved in attentional modulation of odor processing in the OT, we unilaterally injected rats with a retrograde AAV expressing GFP in the OT. We identified GFP-labeled neurons in the medial prefrontal cortex (mPFC), including the prelimbic cortex and infralimbic cortex. GFP-labeled neurons were primarily found in layer 5, consistent with mPFC projections to other regions of the ventral striatum. These results indicate that the mPFC sends direct projections to the OT, and suggest that OT-projecting mPFC neurons may play a role in attentional modulation of odor-evoked activity in the OT. To investigate this possibility, we recorded local field potentials (LFPs) from the mPFC and OT while rats performed a task that requires selective attention to odors. This design allows us to compare LFP dynamics and coherence between the mPFC and the OT as rats attend selectively to odors. Future work will further investigate the importance of OT-projecting mPFC neurons for attention to odors.

Disclosures: H.L. Cansler: None. **D.W. Wesson:** None. **E.E. in 't Zandt:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.06/K23

Topic: D.05. Olfaction and Taste

Support: CIHR Research Project Fund PJT-162124 to Q.Y.
Memorial University Seed, Bridge and Multidisciplinary Fund to Q.Y., C.W.H., and G.M.M.
Dean's Innovation Fund to Q.Y., C.W.H., and G.M.M.
Dean's Transition Fund to Q.Y.

Title: Differential effects of phasic and tonic locus coeruleus activations on odor discrimination and valence learning

Authors: *S. TORRAVILLE[1], A. GHOSH[1], T. OMOLUABI[1], F. MASSAELI[1], V. STRONG[1], K. POWER[1], O. AUDU[1], X. CHEN[1], G. M. MARTIN[2], C. W. HARLEY[2], Q. YUAN[1];
[1]BMS-Neuroscience, [2]Psychology, Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: The pontine nucleus locus coeruleus (LC) is the main source of norepinephrine (NE) in the brain. It has important roles in arousal, learning, emotional regulation, and other functions. LC neurons have two distinct firing modes, phasic and tonic. Phasic bursts of high frequency activity (10-20 Hz) have been associated with focused attention, cortical encoding of salience, and high utility. Tonic firing has been associated with inattention at low frequencies (0.5-2 Hz) and anxiety at high frequencies (5-10 Hz). In this study, we investigated whether tonic and phasic LC spiking patterns differentially control odor discrimination and valence learning. Two-month-old tyrosine hydroxylase - Cre (TH-Cre) rats underwent bilateral stereotaxic infusion surgery of AAV-DJ-EF1a-DIO-hChR2(H134R)-mCherry into the LC. Control rats were infused with AAV-DJ-EF1a-DIO-mCherry. Optical fiber cannulae were implanted one month later. Four different stimulation patterns were used: long phasic (10 Hz, 10s on, 20s off), brief phasic (3 pulses at 10 Hz every 2s), 10 Hz tonic and 25 Hz tonic. General behavioral assessments showed increased rearing occurred with 10 Hz phasic LC activation, whereas 25 Hz tonic activation induced significant immobility. In a go/no-go olfactory discrimination learning task using food as reward, rats underwent dissimilar and similar odor discrimination (DOD and SOD). DOD was given without light while light stimulation accompanied SOD learning. Despite similar DOD learning baselines, ChR2-expressing rats learnt the SOD faster when given 10 Hz phasic patterns, but not with the 10 Hz tonic pattern. In odor valence tasks, rats were light stimulated in one of two odorized T-maze arms. Real-time odor preferences (time spent in each arm) and conditioned odor preferences (rats light-activated in one odorized arm during training were tested without light activation). Ten Hz phasic activation significantly increased the time spent in the

light activated odorized arm in real time with a trend for preference for the conditioned odor in the conditioned task. In contrast, 25 Hz tonic light activation induced a conditioned aversive odor response. These experiments highlight critical differential roles of LC activation patterns in learning and affective behavior.

Disclosures: **S. Torraville:** None. **A. Ghosh:** None. **T. Omoluabi:** None. **F. Massaeli:** None. **V. Strong:** None. **K. Power:** None. **O. Audu:** None. **X. Chen:** None. **G.M. Martin:** None. **C.W. Harley:** None. **Q. Yuan:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.07/K24

Topic: D.05. Olfaction and Taste

Support: NIH R01 DC016289
NIH R01 DC014453
Harvard University QBio Initiative Award

Title: Olfactory evidence accumulation in mice

Authors: *H. WU[1,2], S. JAYAKUMAR[2], J. F. R. GRIMAUD[2], J. D. ZAK[2], P. MASSET[2], V. KAPOOR[2], V. N. MURTHY[2,3];
[1]Chem. & Chem. Biol., [2]Mol. & Cell. Biol., [3]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Olfaction-based navigation is important for the survival of a large variety of animal species. In nature, odor cues from distant objects are usually sparse and highly fluctuating due to turbulence. The statistics of odor concentration over time yields much more reliable information about the location of an object than the transient odor concentration at any given moment. Our research goal is to study how an animal interprets time-varying odor concentration, when the concentration statistics is important for decision-making. We present a new behavioral experiment where mice make decisions based on fluctuating in odor concentration over time. A custom-built device allowed us to deliver pulses of odors (~50 ms wide) at different rates. We show that mice can be trained to perform well when differentiating stochastic odor stimuli with different underlying statistics (average pulse rates) presented over several seconds. Performance was nearly perfect (~90% correct) when mice differentiated between stimuli with large differences in average pulse rate, and degraded when rate differences diminished. The discrete nature of the sniffing behavior of mice gives us a special opportunity to investigate how active, discrete sampling of a continuously-varying environmental cue affects the neural and conceptual interpretation of the cue. With calcium imaging of the olfactory bulb, we found that the neural activity in the glomeruli of olfactory bulb are highly-modulated by both the concentration of the

odor and the phase of the sniffing cycle. Ongoing experiments are aimed at uncovering the behavioral and neural signatures of evidence accumulation in this novel olfactory task.

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Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.08/K25

Topic: D.05. Olfaction and Taste

Support: NIH Grant R01DC015426
NIH Grant T32MH067564

Title: Tuning of ensemble responses in human olfactory cortex

Authors: *V. SAGAR, T. KAHNT;
Neurol., Northwestern Univ., Chicago, IL

Abstract: The human olfactory system transforms chemical information from odor molecules into neural representations of perceptual and motivational features. Neural activity in primary olfactory areas can be used to decode information about odor stimuli but it is unclear which features of odors are encoded in these brain regions that allow us to decode odor information. Here we address this question using functional magnetic resonance imaging (fMRI) and neural encoding models that explicitly model the tuning of ensemble responses in individual voxels. We recorded 24 hours of fMRI data from a single human subject while she repeatedly smelled 160 monomolecular odors. We modeled odor-evoked fMRI responses in primary olfactory areas as a linear combination of perceptual (fruity, bakery, fishy, minty, etc.) and chemical basis functions. Importantly, we used these models to make out-of-sample predictions about the fMRI responses evoked by odors that were not used to train the model. Preliminary analyses show that accuracy for this out-of-sample prediction was significant in several olfactory areas, including piriform cortex, amygdala, entorhinal cortex and orbitofrontal cortex. This shows that neuronal populations in these areas are tuned to a linear combination of perceptual and chemical properties of the odors. Moreover, a clustering analysis of the weights assigned to perceptual basis functions revealed a large-scale topography of perceptual vs. motivational features, in conjunction with locally distributed encoding of qualitative perceptual features in the piriform cortex. Our results show that encoding models can be used to reveal the tuning properties of neuronal ensembles in the human olfactory cortex.

Disclosures: V. Sagar: None. **T. Kahnt:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.09/K26

Topic: D.05. Olfaction and Taste

Support: W911NF-16-1-0316
W911NF-18-1-0292

Title: A novel computer vision tool to infer fish behavior in response to odor stimulation

Authors: *S. BANERJEE[1], W. J. SCHEIRER[1], L. LI[2];
[1]Computer Sci. & Engin., [2]Biol. Sci., Univ. of Notre Dame, Notre Dame, IN

Abstract: The engagement of sexual behaviors in animals is regulated by several factors that include gene expression, hormone circulation, and multi-sensory information integration. Sexual behavior, or more precisely mating-like behavior in fishes can be measured by quantifying their movement through location, speed, direction and swim pattern in the presence of another fish, same or different gender.
In zebrafish, when a male and a female are placed in the same container, they show mating-like behaviors irrespective of whether they are kept together or separated by a net. No mating-like behaviors are observed when same-sex animals are put together. Li (Li et al., 2017) demonstrated that through the olfacto-visual centrifugal pathway, activation of the terminalis nerve in the olfactory bulb increases GnRH signaling in the brain and triggers mating-like behaviors between
males. This behavior is however, repressed in zebrafish mutants or wild-type fish in which the olfacto-visual centrifugal pathway is either genetically impaired or chemically ablated. Together, the data suggests that the combination of olfactory and visual signals alter male zebrafish's mating-like behaviors via GnRH signaling.
To characterize the sexual behavioral changes due to olfactory signals, the swimming behaviors of the fish is videotaped over time after the application of methionine to the water. Biologists, currently skim through these videos and take notes at certain time intervals about the relative position of fish in water tank with respect to a grid placed just below it. In this work, we propose to automate this entire process by using tools from computer vision. The overall objective of this work is to create a generalized tool for neuroscience to predict animal behaviors from videos using state-of-the-art deep learning models, with the dual goal of advancing understanding in biology and building more robust and powerful artificial information processing systems.
Reference:
1. Li, L., Wojtowicz, J. L., Malin, J. H., Huang, T., Lee, E. B., & Chen, Z. (2017). GnRH-mediated olfactory and visual inputs promote mating-like behaviors in male zebrafish. *PloS one,*

12(3),
e0174143.

Disclosures: **S. Banerjee:** None. **W.J. Scheirer:** None. **L. Li:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.10/K27

Topic: D.05. Olfaction and Taste

Support: PROGRAMA UNAM-DGAPA-PAPIIT-IN224417

Title: Characterization of the olfactory and cardiac response in the establishment of hierarchical order in crayfish

Authors: *I. HERNANDEZ-PRIOR, K. MENDOZA-ANGELES, J. HERNANDEZ-FALCON;
Dept. de Fisiología, Univ. Nacional Autónoma De México, Ciudad de México, Mexico

Abstract: Sensory systems provide an organism with information about both the inner and the external environments. This information is then processed in the brain by high-order integration centers to produce an adequate response. Agonistic behavior and memory recognition in crayfish seem to depend mainly on olfactory information carried out by a compound(s) present in urine streams, that is released during social interactions. This putative compound(s) has not been identified but the lack of olfactory information, by the blockade of olfactory receptors or the urine release through the nephropores, induce long-lasting fights between contenders, even after the hierarchical order was previously established. The main goal of this work was to identify brain, chemoreceptor and cardiac electrical responses during the establishment of dominant-submissive hierarchical order in adult crayfish.
We used adult male crayfish implanted, under cold anesthesia, with a brain electrode, on the olfactory lobe, and simultaneously a peripheral electrode that recorded chemoreceptor activity from the antennule and cardiac activity. In triads of crayfish we videotaped agonistic interactions until a hierarchical order was established. Then we applied urine from the dominant animal on the recorded antennule of a submissive one and recorded the central and peripheral response as well as the behavior of the animal.
Urine from the dominant animal induced a bimodal electrical response, an immediate reduction in the electrical activity of the brain and the antennule, followed by bursts of discharges with variable duration. The cardiac electrical activity presented a great increase in frequency and voltage; this increase was long lasting with duration greater than 15 minutes. Behaviorally, the stimulated animal increased locomotor activity and showed even an escape response. Urine from

a submissive crayfish applied to a dominant one, was accompanied by less intense electrophysiological and behavioral changes. These results point out to the relevance of the urine released during agonistic encounters that allow the recognition of conspecifics, the maintenance of hierarchical status, and the increase in cardiac activity in a probably autonomic-like response.

Disclosures: I. Hernandez-Prior: None. **K. Mendoza-Angeles:** None. **J. Hernandez-Falcon:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.11/K28

Topic: D.05. Olfaction and Taste

Support: JSPS KAKENHI Grant Number 18J21358
JSPS KAKENHI Grant Number 16H02061
JSPS KAKENHI Grant Number 16K14557
JSPS KAKENHI Grant Number 25135708
Takeda Science Foundation
Narishige Neuroscience Research Foundation

Title: Behavioral scene-selective activity of ventral tenia tecta neurons

Authors: *K. SHIOTANI[1,2], Y. TANISUMI[1,2], K. MURATA[3], J. HIROKAWA[1], K. MORI[4], Y. SAKURAI[1], H. MANABE[1];
[1]Grad. Sch. of Brain Sci., Doshisha Univ., Kyoto, Japan; [2]Res. Fellow of Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Fac. of Med. Sci., Univ. of Fukui, Fukui, Japan; [4]The Univ. of Tokyo, Tokyo, Japan

Abstract: The ventral tenia tecta (vTT) is an area of the olfactory cortex located in the ventromedial part of the olfactory peduncle in mice. vTT neurons receive olfactory sensory signals from axons of mitral cells in the olfactory bulb. However, little is known about how the vTT anatomically connects to among other olfactory cortical areas and other brain regions and how the vTT translate the olfactory sensory information into behavioral responses. First, we performed anterograde and retrograde tracing from the vTT and observed that the vTT has reciprocal connections with many olfactory areas, including the anterior olfactory nucleus and anterior and posterior piriform cortex. We also found that the vTT received direct inputs from the medial prefrontal cortex. These results suggest that vTT neurons can be driven not only by the olfactory bulb afferent input that conveys odor information but also by association fiber inputs from many olfactory areas and top-down inputs from the medial prefrontal cortex. To determine whether vTT neurons encode behavior-specific signals during goal-directed behavior, we

recorded the individual neuronal responses in the vTT of freely moving, awake mice that performed learned odor-guided Go/No-Go discrimination task. Many neurons in the vTT changed their firing rates not only during odor exposure but also during approaching, anticipating, and getting reward. We found that the firing pattern of individual vTT neurons had reproducible behavioral correlates, so that the environmental and behavioral scene the mouse encountered during the learned behavior was the major determinant of when individual vTT neurons fired maximally. These results indicate that different groups of vTT neurons are activated in different scenes, and suggest that the processing of olfactory sensory information is handled by different scene neurons during distinct scenes of learned odor-guided behavior.

Disclosures: K. Shiotani: None. **Y. Tanisumi:** None. **K. Murata:** None. **J. Hirokawa:** None. **K. Mori:** None. **Y. Sakurai:** None. **H. Manabe:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.12/K29

Topic: D.05. Olfaction and Taste

Support: R01 DC011286
R01 DC014723
NSF BRAIN 1555880

Title: Mouse detection of fluctuating odors based on intermittency

Authors: *A. GUMASTE[1,3], **M. IZYDORCZAK**[3], E. CONNOR[4], K. L. BAKER[3,2], K. NAGEL[5], J. CRIMALDI[4], J. V. VERHAGEN[6,2];
[1]Interdepartmental Neurosci. Program, [2]Neurosci., Yale Univ., New Haven, CT; [3]John B Pierce Lab., New Haven, CT; [4]Civil, Environmental, and Architectural Engin., Univ. of Colorado, Boulder, CO; [5]Neurosci. Inst., NYU Langone Med. Sch., New York, NY; [6]John B. Pierce Lab., New Haven, CT

Abstract: Odor-based navigation is critical to animal survival as animals depend on olfactory cues to locate food sources, find mates, and avoid predators. Odors in nature are often carried by turbulent air flow, producing plumes with complex spatiotemporal structure. In large naturalistic environments, odor plumes become filamentous and characterized by odor fluctuations with highly varying intermittency. Such complex odor information must be actively sampled through sniffing, integrated by the olfactory system, translated into odor identity, and ultimately, drive behavior. However, little is known about how animals actively sample fluctuating odor stimuli to detect relevant stimulus properties and how this sniff-dependent odor sampling is encoded by olfactory sensory neurons. In a collaborative effort, we have designed a go-nogo task that

implements turbulent odor plumes, characterized by Planar Laser Induced Fluorescence (PLIF), as stimuli. More specifically, we have trained water-regulated OMP-GCaMP6f mice to discriminate between dynamic naturalistic and synthetic (square-wave) fluctuating odor sequences varying in intermittency. If animals lick following the presentation of a S+ stimulus (a stimulus of low intermittency), they receive a water reward, however if they lick following the presentation of a S- stimulus (a stimulus of intermittency value greater than 0.2), they receive an time out in the form of an increased inter-trial interval. As animals are performing the task, we simultaneously record sniffing responses as well as image neural activity of mature olfactory sensory neurons in the dorsal olfactory bulb. Using this behavioral paradigm, we have found that animals can discriminate different levels of intermittency and we have expanded the current understanding of the sniffing strategies implemented by animals to detect fluctuating odors. Additionally, we have explored how neural encoding of fluctuating stimuli depends on sniffing patterns in both anesthetized and awake animals.

Disclosures: **A. Gumaste:** None. **M. Izydorczak:** None. **E. Connor:** None. **K.L. Baker:** None. **K. Nagel:** None. **J. Crimaldi:** None. **J.V. Verhagen:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.13/K30

Topic: D.05. Olfaction and Taste

Support: NIH R01 DC014367

Title: A geometric framework for odor learning and representation

Authors: *J. A. COOK[1], T. A. CLELAND[2];
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Abstract: The structure of olfactory categorical representations is fundamentally dependent on learning. Plasticity within the olfactory bulb has been proposed to govern the construction of these experience-dependent representations. Recent computational work from our group demonstrates how bulbar circuitry can underlie the rapid learning of probabilistic categorical representations of meaningful odors and the subsequent recognition of these odors under strongly interfering conditions. We sought to embed this emerging theoretical framework within an analytical model of odor learning, incorporating the construction of hierarchical representations as well as ancillary psychophysical phenomena such as generalization gradients and the speed-accuracy tradeoff.
We construct a generalized framework for olfactory representation, learning, and perception using the tools of real differential geometry and abstract algebra. We use the convenient

geometry of locally Euclidean spaces and their associated algebraic structures, finding that the incorporation of plasticity renders obsolete any possible advantages of hyperbolic geometry. Beginning with the space of all possible physical inputs to the olfactory system (R-space), we transform this input space into a smooth, pliable perceptual output, incorporating a dynamic model for perceptual learning that utilizes the set of all (cross-)sections from R-space to a physical-perceptual transition space, which in turn modifies the perceptual space. This perceptual S-space develops the hierarchical categories of learned odors atop an intact physical similarity metric, thereby embedding interpretable depictions of stimulus generalization and the speed-accuracy trade-off. As a corollary of this construction, we find that geometries that fix curvature, whether Euclidean, hyperbolic, or elliptical, all are insufficient to describe the dynamics of olfactory perceptual space as they are, quite literally, too rigid.
Odors encountered in the wild are usually embedded within strongly interfering chemosensory backgrounds, yet are readily recognized and identified by animal olfactory systems. To incorporate the recognition of such occluded odors within this framework, we investigate flows along vector fields associated to both S- and R-spaces. Synaptic plasticity and adult neurogenesis in the olfactory bulb, which enable the identification of occluded odors in biomimetic networks, here act to systematically modify these vector fields, drawing odorant signatures towards learned categorical representations.

Disclosures: **J.A. Cook:** None. **T.A. Cleland:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.14/K31

Topic: D.05. Olfaction and Taste

Support: CIHR Grant

Title: Pheromone-mediated vocalizations resist habituation

Authors: *A. E. PHILIPP-MULLER, J. KIM;
Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Male mice produce ultrasonic vocalizations in the company of females and can later produce similar calls in the presence of female pheromones alone. We studied male habituation to female pheromones in the absence of a live female. Eight male subjects were pre-exposed to females and then housed in isolation. The males were then exposed to female urine containing female sex-pheromones for 5 minutes every 12 hours until they returned to baseline. Recognition of the female pheromone was measured in terms of the number of mating calls produced per minute by the males during each session. Subjects called significantly more often in the presence

of female pheromone, however their call-rate did not differ based on experience. The results suggest that the drive to produce ultrasonic vocalizations in response to female pheromones is an automatic, potentially subcortical process.

Disclosures: A.E. Philipp-Muller: None. **J. Kim:** 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.; CIHR.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.15/K32

Topic: D.05. Olfaction and Taste

Support: NIH Grant RO1MH101293
NIH Grant RO1DC013090

Title: Tailshock evokes widespread, spatially and temporally patterned neural activity in the mouse olfactory bulb *in vivo*

Authors: *K. A. PERKINS, Jr, J. P. MCGANN;
Behavioral and Systems Neuroscience: Psychology, Rutgers Univ., New Brunswick, NJ

Abstract: Odor-cued fear conditioning induces neuroplasticity in olfactory sensory neurons terminals and periglomerular interneurons in the olfactory bulb (Kass, Rosenthal et al. 2013; Kass and McGann 2017).
This requires convergence of olfactory sensory information with shock-evoked neural activity. Here, we used *in vivo,* wide field optical neurophysiology to test whether a tailshock could evoke activity in various neuronal populations in the olfactory bulb, as visualized through a cranial window in naïve anesthetized mice. Reporter mice were generated that selectively expressed the fluorescent calcium indicator GCaMP6f in olfactory sensory neurons (OSNs), GAD65-expressing periglomerular (PG) interneurons, short axon (SA) cells, or mitral cells by crossing appropriate cre recombinase-expressing driver lines with the Ai95 reporter line. In each of these lines, odorant presentation evoked the expected focal activity in an odor specific subset of olfactory bulb glomeruli. Tailshocks more varied responses. In the SA cell population, tailshocks evoked large, focal GCaMP signals in a subset of distinctive glomeruli, sometimes evolving over several seconds to engage additional glomeruli. In the PG cell population, tailshocks evoked focal glomerular signals amid a more diffuse, bulb-wide increase in fluorescence. The population of mitral cells exhibited diffuse increases in fluorescence throughout the bulb following each tailshock. In all cases, the neural response in the bulb was timed to the inhalation following the

tailshock, and elimination of intranasal airflow eliminated responses to tailshock in PG, SA, and mitral cell populations ipsilateral to a unilateral nasal plug or bilaterally following tracheotomy. This suggested that tailshock evokes bulbar activity by enhancing the inhalation-evoked output of the OSNs. However, OSN populations exhibited little or no response to tailshock (measured at their synaptic terminals) despite the often-pronounced shock-evoked inhalation and the necessity of peripheral airflow for shock-evoked activity in the PG, SA, and mitral cells. We conclude that tailshock evokes widespread activity in the olfactory bulb, even under anesthesia, and this activity may be gated by peripheral input.

Disclosures: K.A. Perkins: None. **J.P. McGann:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.16/K33

Topic: D.05. Olfaction and Taste

Support: Dirección General de Asuntos del Personal Académico (DGAPA) de la Universidad Nacional Autónoma de México (UNAM) GIVEN TO D.R-S
DGAPA-PAPIIIT IN224019 and IA206919
CONACYT (GRANT NUMBER 294541)

Title: Reduced olfactory perception to isoamyl acetate in streptozotocin-induced diabetic female rats

Authors: *D. REBOLLEDO-SOLLEIRO[1], J. E. RIOS-CARRILLO[1], I. CRUZ-GUTIERREZ[1], A. ESPINOZA-SALGADO[1], R. C. ZEPEDA[2], H. SOLLEIRO-VILLAVICENCIO[1], G. ROLDAN-ROLDAN[1];
[1]Fisiologia, Facultad de Medicina, Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; [2]Ctr. De Investigaciones Biomedicas, Univ. Veracruzana, Jalapa, Veracruz, Mexico

Abstract: Diabetic patients commonly develop olfactory dysfunctions. However, clinical results are controversial and preclinical studies are scarce. There are only two reports demonstrating that streptozotocin (STZ)-induced diabetic (SID) male rats show reduced odor discrimination and decreased olfactory sensitivity, as measured in the buried food pellet and novel object discrimination tests, respectively. Since several structures related with olfaction exhibit sexual dimorphism, it is important to explore whether SID females also have impaired olfactory function. To induce hyperglycemia, STZ was administered (50mg/kg in citrate Buffer 0.1M, pH 4.5, i.p.) in two consecutive days to female young-adult Wistar rats; controls were injected with vehicle. Ten days after STZ or vehicle administration, animals were submitted to the conditioned odor aversion (COA), in which an aversive stimulus that provokes visceral malaise (LiCl 2%

BW, i.p.) is paired with the consumption of an odorant diluted in water. We used two odorants: isoamyl acetate (IA), an odor that represent a food item and activates the main olfactory system; 2-heptanone (2-Hep), a pheromone that activates the accessory olfactory system. To determine olfactory perception, we used six concentrations ($5x10^{-5}$-$5x10^{-11}$) of IA/2-Hep, each of them tested in consecutive days. We determined the index of preference, by calculating the volume of IA/2-Hep solution consumed x 100 / the total volume consumed (AI solution + water). This index allowed us to know whether the animals were properly conditioned, implicating that they perceived the odorant correctly. The data were analyzed with Student T-test to compare results between experimental groups. Our results show that all experimental subjects (euglycemic and hyperglycemic) avoided odorant consumption (AI/2-Hep) after COA. Moreover, odor recognition was abolished when using less concentrated solutions. Notably, hyperglycemic females showed reduced capacity to recognize IA. No statistical changes were observed in olfactory discrimination using 2-Hept, indicating specific hyposmia. Finally, our data are in line with clinical observations made in patients with DM and SID male rats. These results also suggest that STZ treatment (which provokes oxidative stress in the Central Nervous System) affects differentially the main and accessory olfactory systems.

Disclosures: D. Rebolledo-solleiro: None. **J.E. Rios-carrillo:** None. **I. Cruz-gutierrez:** None. **A. Espinoza-salgado:** None. **R.C. Zepeda:** None. **H. Solleiro-villavicencio:** None. **G. Roldan-roldan:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.17/K34

Topic: D.05. Olfaction and Taste

Title: Behavioral decision making for predator defensive behaviors in mice

Authors: *Q. T. NGUYEN, S. HAGA-YAMANAKA;
Univ. of California Riverside, Riverside, CA

Abstract: Defensive behaviors in the presence of predatory cues are expected to be driven by innate neurological activation. Despite the innate feature, prey animals exhibit varieties of defensive behavioral outputs depending on the context such as availability of flight, distance from predators, and immediacy. For example, prey animals exhibit flight or freeze behaviors for acute dangers and risk assessment behaviors towards potential dangers in the environment. Neural mechanisms underlying this innate decision-making process has not been fully understood yet. Predatory cue detection is optimal with the summation of various sensory modalities in nature, yet, exposure to olfactory-mediated predator cues (predator odors) are sufficient to yield those different types of defensive behaviors in some prey species such as mice.

Thus, in this study, we examined mouse defensive behavioral responses towards predator odors collected and presented in varieties of contexts. Neural activations associated with the behavioral outputs were also examined by *post-hoc* c-Fos immunostaining after the behavioral tests. We observed that mice predominantly exhibited predicted behavioral output to a predator odor sample in a specific context. The number of c-Fos-immunoreactivity in the neural substrates involved in defensive behaviors are different among the animals showing different defensive behaviors. Moreover, to examine whether the conditions of recipient animals affect the behavioral-decision process or not, we compared behavioral responses as well as neural activation in mice of different strains, sexes, and ages. We found female mice were more inquisitive, suggesting possible variations in speed of odor detection between sexes. Furthermore, we discovered variations in freezing duration of the animals when presented with different predator cues. Our results suggest that mice can discriminate predator odors in specific contexts such as immediacy, while exhibiting detectable differences in freeze behavior latency between mouse strains, sexes, and ages. The defensive neural circuitries distinguish the sensory inputs and induce specific behavioral responses that is appropriate for each context. Moreover, the behavioral decision could be modulated in mice in different conditions. Our goal is to identify the neural mechanism underlying this innate decision making in predator defensive behaviors in mice.

Disclosures: Q.T. Nguyen: None. **S. Haga-Yamanaka:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.18/K35

Topic: D.05. Olfaction and Taste

Support: NIH R01NS104818

Title: Modulation of olfactory learning and processing by baseline gustatory cortex inputs

Authors: *B. N. BALLINTYN[1], D. B. KATZ[2], P. MILLER[1];
[2]Dept Psychol, [1]Brandeis Univ., Waltham, MA

Abstract: The integration of information from multiple sensory modalities is important for generating accurate representations of external objects. In our previous work, we have found behavioral and electrophysiological evidence for functional interactions between gustatory (GC) and olfactory (OC) cortices. In social-transmission of food preference (STFP) experiments, recognition of an odor on the breath of another rat enhances preference for the corresponding food. However, this preference is lost if GC activity is silenced at the time of transmission (learning) or at the time of the preference test (this behavior has also been seen in conditioned

odor preference experiments). Surprisingly, however, if GC activity is silenced at both times, then the expression of the learned preference is normal. This suggests that baseline GC activity acts as a contextual modulator of OC odor responses and indeed this idea has been validated by past electrophysiological experiments. This leaves open the question of how baseline activity in one sensory region can modulate responses in another while preserving functionality. In this study, we use computer simulations of an OC network of spiking neurons (with odor and GC inputs) and an optimization procedure to assess whether 3 different OC connectivity motifs (random, clustered, and gaussian) and 2 input motifs (random, or following a gradient) are able to reproduce the behavioral and electrophysiological observations from experiment. Importantly, in our simulations we assume no stimulus dependent structure since the odors used in the STFP experiments are novel to the animal. We find that model OC networks that most closely match the experimental constraints receive spatially segregated inputs from GC and OB along gradients. Additionally, inhibitory OC neurons must be at least as excited as excitatory OC neurons by GC inputs in order to reproduce the desired behavior. In summary, we have shown through simulations that an observed double dissociation in behavior causes models of the circuitry responsible for the behavior to be highly constrained in a manner that is compatible with the measured neural responses.

Disclosures: **B.N. Ballintyn:** None. **D.B. Katz:** None. **P. Miller:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.19/K36

Topic: D.05. Olfaction and Taste

Support: DARPA HR0011-18-2-0024

Title: The role of the olfactory bulb respiratory rhythm in coordinating neocortical and limbic system oscillations for sensory cognition

Authors: *A. SHERIFF[1,2], L. M. KAY[1,2];
[1]Inst. for Mind and Biol., Chicago, IL; [2]Psychology, The Univ. of Chicago, Chicago, IL

Abstract: The olfactory nerve excites olfactory bulb (OB) neurons with each inhalation producing a respiratory-locked rhythm that dynamically coordinates OB neural oscillations. Nasal respiration modulates neocortical and limbic oscillations in humans underlying cognitive processes, including memory. In rodents, the hippocampus (HPC) and OB both exhibit HPC theta and the OB sniff rhythm during locomotion at overlapping frequencies, suggesting complementary functions. To test the functionality of the OB-driven respiratory rhythm on network interactions that underlie cognition, we performed olfactory bulbectomy (OBx) in young

Long Evans rats just post-weaning as a model of congenital anosmia. This was followed many weeks later by implantation of probes to measure nasal respiration and electrodes to measure local field potentials in piriform cortex (PC), dentate gyrus and CA1 of HPC, and visual cortex (V1M). Recordings were conducted while rats foraged in both dark and dimly lit environments with olfactory or visual spatial cues, respectively. Data from intact rats show high coherence at OB sniff frequency between distal brain systems while they forage in both conditions. Respiration tended to be faster than HPC theta. Neural populations oscillating at high phase coherence with the OB (*e.g.*, PC-OB) were mostly in respiratory frequency while coherence with HPC (*e.g.*, PC-DG) tended to be at HPC theta frequency. OBx rats show lack of respiratory coupling and altered coherence patterns between areas in visual versus olfactory foraging contexts, along with differences in network directionality as measured by Granger Causality, especially in interactions with the PC. Further behavioral testing includes a nasal trigeminal discrimination task (Go/No-Go). OBx and intact rats learned at similar rates on this task. Changes in behavioral strategy, patterns of coherence, and directionality of network patterns were assessed during initial acquisition of rule-transfer and reversal learning. Findings here aim to differentiate the roles of nasal respiratory and HPC theta oscillations in coordinating sensory and limbic systems, and to elucidate potential compensatory mechanisms of congenital anosmia.

Disclosures: **A. Sheriff:** None. **L.M. Kay:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.20/K37

Topic: D.05. Olfaction and Taste

Support: NIH/NINDS R24 NS098536
Boston University Neurophotonics Center

Title: Bilateral imaging of sensory dynamics during odor-guided navigation in freely behaving mice

Authors: D. P. LEMAN[1], I. A. CHEN[2], W. W.-S. YEN[1], J. CLEVENGER[2], N. PERKINS[2], L. KRETSGE[1], Y. COHEN[1], W. A. LIBERTI, III[3], K. KILIÇ[2], A. CRUZ-MARTIN[1], T. J. GARDNER[1], T. M. OTCHY[1], *I. G. DAVISON[1];
[1]Dept. of Biol., [2]Dept. of Biomed. Engin., Boston Univ., Boston, MA; [3]Univ. of California Berkeley, Berkeley, CA

Abstract: In natural contexts, freely behaving animals guide ongoing actions by actively sampling sensory cues from their surroundings. In the olfactory system, ethological behaviors are informed by both the identity of chemical signals and spatial intensity gradients indicating their

location. While the way that odor identity is encoded by sensory maps in olfactory bulb has been extensively studied in head-fixed animals, little is known about the neural signals that direct spatial behaviors such navigation. Progress has been limited by a lack of tools for capturing the bilateral activity patterns likely to contribute to localization. Here, we use a novel system for large-scale Ca^{2+} imaging in freely moving mice to visualize sensory-driven activity during navigation towards an odor source. While miniaturized, head-mounted fluorescence "miniscopes" have enabled imaging during naturalistic behaviors, the gradient-index optics of current systems restrict effective imaging areas to $<1mm^2$. First, we present a new wide-field miniscope design that expands field of view by an order of magnitude to approximately 10 mm^2, while maintaining single-neuron resolution and weighing less than 4.5 grams. Second, we use the wide-field system to compare the dynamics of sensory responses across hemispheres of the main olfactory bulb of mice engaged in an odor-based localization task. Active investigation drives complex spatio-temporal activity patterns in mitral/tufted cells, including distinct maps of glomerular activation corresponding to different odorants as previously described. In addition, we find that active investigation generates robust differences in timing and intensity of responses between left and right olfactory bulbs occurring within a single sniff cycle. The olfactory system thus encodes robust 'stereo' cues that provide a potential basis for guiding movement. To test this idea, ongoing experiments are applying high-speed behavioral tracking to correlate changes in movement with neural activity as mice localize odor sources in space. This approach promises to provide insight into the sensorimotor strategies that underlie naturalistic spatial behaviors.

Disclosures: **D.P. Leman:** None. **I.A. Chen:** None. **W.W. Yen:** None. **J. Clevenger:** None. **N. Perkins:** None. **L. Kretsge:** None. **Y. Cohen:** None. **W.A. Liberti:** None. **K. Kiliç:** None. **A. Cruz-Martin:** None. **T.J. Gardner:** A. Employment/Salary (full or part-time):; Neuralink, Inc.. **T.M. Otchy:** None. **I.G. Davison:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.21/K38

Topic: D.05. Olfaction and Taste

Support: NIH Grant 1R01DC017149-01A1

Title: Vomeronasal sensory neurons undergo identity drifts and functional ambiguity after loss of Tfap2e (AP-2e)

Authors: *J. M. LIN, P. E. FORNI;
Biol., SUNY, Univ. at Albany, Albany, NY

Abstract: The molecular mechanisms controlling the definition and maintenance of neuron-specific gene batteries instruct neuron function and circuitry formation remain largely unknown. To study this, we have utilized the rodent accessory olfactory subsystem due to its ability for neurogenesis throughout life, distinct axonal targeting, predictable neuronal activation patterns, and the highly characterized behavioral outputs following exposure to specific stimuli. Vomeronasal sensory neurons (VSNs) are generated from a common pool of progenitor cells and are divided into apical and basal neurons which express one or two vomeronasal receptors (VRs) from different families which detect certain pheromones and transduce that signal to the anterior (aAOB) or posterior (pAOB) accessory olfactory bulb (AOB). It has been previously shown that the transcription factor Tfap2e (AP-2ε) is necessary for the normal maturation and identity maintenance in basal VSNs. AP-2ε loss-of-function can lead to the gain of apical characteristics, such as the apical transcription factor, Meis2, and some V1r receptors, suggesting some degree of transdifferentiation *in vivo*. To test the functionality of the putative transdifferentiated neurons in the AP-2ε KOs, we exposed adult male mice to female-soiled bedding, which selectively activates V1r expressing apical VSNs. We checked for activation in the VSNs by immunostaining for the phosphorylated ribosomal subunit S6 (pS6) and found activated neurons throughout the epithelium in the AP-2ε KO, while in WTs, pS6+ neurons were only found in the apical region of the epithelium. We also checked for activated areas in the brain by immunostaining for cFOS, and found ectopic activation of the pAOB in the AP-2ε KO mice. These data suggest that the gain of V1r expression can lead to aberrant innervation and activation of the AOB. We further analyzed the organization of the AOB and axonal targeting of the putative transdifferentiated neurons by using immunostaining paired with AP-2ε genetic lineage tracing in KOs and heterozygous controls. Quantifications of number and size of the glomeruli using VGlut2 revealed that glomeruli in the pAOB have lost definitive borders and have merged into fewer, but larger, glomerular areas, suggesting the loss of targeting specificity to the pAOB. While glomerular formation in the aAOB remains largely unchanged, we observed a significant increase in the average size of the glomeruli. Additionally, we observe ectopic innervation of AP-2ε lineage-traced axons innervating glomeruli in the aAOB, indicating that some neurons are capable of fully switching identity and synaptic targets in the absence of functional AP-2ε.

Disclosures: J.M. Lin: None. **P.E. Forni:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.22/K39

Topic: D.05. Olfaction and Taste

Support: NIH Grant 5R03DC14788

Title: Odor detection and discrimination by immature mouse olfactory sensory neurons *in vivo*

Authors: *J. HUANG[1], B. LIU[2], J. AVON[3], R. MUGGLETON[2], C. E. CHEETHAM[3];
[1]Ctr. for Neurosci., [3]Dept. of Neurobio., [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Mammalian olfactory sensory neurons (OSNs) are generated throughout life in the olfactory epithelium. However, when newly generated OSNs begin to play a functional role in olfaction is poorly understood. Recent studies have shown that odorant receptor expression precedes OSN maturation by several days (Rodriguez-Gil et al., 2015; Hanchate et al., 2015; Tan et al., 2015). Using *in vivo* 2-photon calcium imaging, we show that immature OSNs can detect and transduce odor binding: 88% of glomeruli innervated by immature OSN axons responded to at least one odor in a 12-odor panel (n=87 glomeruli, 4 mice). Across the odor panel, responses were smaller in magnitude in Gγ8-GCaMP6s mice (αF/F [mean ± SD] 29 ± 47%) than in OMP-GCaMP6s mice (αF/F 58 ± 69%; P<0.001, 2-way ANOVA).
We have shown previously that immature OSNs form synapses and can evoke robust stimulus-locked firing of OB neurons (Cheetham et al., 2016). Here, we used optogenetics and electrophysiology in OB slices to show that immature OSNs form monosynaptic connections with both mitral cells (MCs) and tufted cells (TCs). Following optogenetic stimulation of immature OSN axons, we detected a monosynaptic EPSC in 20% of MCs (n=10 cells) and 30% of TCs (n=10 cells). In contrast, following optogenetic stimulation of mature OSN axons, monosynaptic EPSCs were detected in 60% (n=10 cells) and 100% (n=10 cells) of MCs and TCs, respectively. This difference may be due to the lower density of immature vs. mature OSN axons in glomeruli. Together, these data demonstrate that immature OSNs can detect odor binding and transduce the signal into action potentials that are transmitted to neurons in the olfactory bulb (OB).
We then used methimazole (MMZ) ablation with short (5-7 day) recovery periods to generate mice that had immature OSNs but lacked all mature OSNs. At 5 days post-MMZ, 33% of mice successfully performed a buried food assay, rising to 67% at 6-7 days post-MMZ. This shows that mice can detect odors using immature OSNs alone. Mice also successfully performed a simple odor discrimination task at 7 days post-MMZ, with habituation and dishabituation being statistically indistinguishable from control mice. Together, these findings suggest that immature OSNs may play a previously unappreciated role in olfaction.

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Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.23/K40

Topic: D.05. Olfaction and Taste

Support: Blakeslee Fund, Smith College

Title: Interactions of DEET and novel repellents with mosquito odorant receptors

Authors: G. G. GRANT[1], R. R. ESTRERA[2], M. J. REGAN[3], *A. C. HALL[1];
[1]Biol. Sci., [2]Neurosci., [3]Biochem., Smith Col., Northampton, MA

Abstract: Insect-borne diseases such as malaria, dengue and yellow fever are responsible for millions of deaths annually. Repellents disrupt interactions between humans and insects, playing a crucial role in providing protection from bites through which diseases are transmitted. The most commonly used active ingredient in topically-applied repellents today is N, N- diethyl-*meta*-toluamide (DEET). Despite its popularity, DEET's mechanism of action has not yet been definitively determined. Previous research indicates that DEET inhibits odor-evoked currents mediated by subsets of heteromeric insect odorant receptors expressed in *Xenopus laevis* oocytes (Ditzen et al, 2008). Odorant activation of the receptor complexes results in non-selective cation fluxes in oocytes.

Based on the premise that repellency may occur through receptor-mediated inhibition of insect olfactory transduction, the present study aims to explore the association between odorant receptor inhibition and repellent potency. This electrophysiological investigation utilized two electrode voltage clamp with the described *X. laevis* oocyte expression system, to determine the effects of 17 novel repellents on odorant receptors from the *Anopheles* mosquito. The tested repellents fell under two categories of chemicals: acylpiperidines and carboxamides (including DEET). The novel repellents have been shown to possess different repellent potencies in previously carried out skin tests with feeding mosquitoes (Katritzky et al, 2008). Results indicated that all repellents, when co-applied at a 300 μM concentration with the odorant, 2-methyl phenol, inhibited currents evoked from a GPROR2 + GPROR7 receptor combination. DEET, with a repellency of 2.5 (protection time in days), inhibited currents by 11.7% ± 3.4% (n = 33). The most potent acylpiperidine repellent, with a repellency of 13.5, inhibited currents by 44.6% ± 5.7%, while the least potent carboxamide, with a repellency of 2.0, inhibited currents by 16.3% ± 2.8% (n = 5). These data suggest a possible relationship between the potency of a repellent and its inhibition of insect odorant receptor activity, which can inform future efforts in the design and discovery of repellents.

Ditzen et al, 2008, Science, 319, 1838-1842
Katritzky et al, 2008, PNAS, 105, 7359-7364

Disclosures: G.G. Grant: None. **R.R. Estrera:** None. **M.J. Regan:** None. **A.C. Hall:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.24/L1

Topic: D.05. Olfaction and Taste

Support: NIDCD R01 DC014367
DARPA HR0011-18-2-0024

Title: Learning transfer from retro- to orthonasal olfaction and odor-evoked local field potential characterization across olfactory routes

Authors: *R. HE[1], K. CHEN[2], L. M. KAY[3];
[1]Dept. of Psychology, [3]Psychology, [2]The Univ. of Chicago, Chicago, IL

Abstract: Grants: NIDCD R01 DC014367 and DARPA HR0011-18-2-0024 to LMK
Odorants can activate the nasal epithelium from two directions, either from the nares via nasal breathing or sniffing (the orthonasal route) or through the mouth during eating or drinking (the retronasal route). We use olfactory discrimination tasks and electrophysiological recording to study the perceptual qualities of two olfactory routes and circuit interactions when given the same chemical input ortho- or retronasally. In Experiment 1, rats were pre-conditioned to odorized solutions delivered only retronasally. We then tested rats in an orthonasal Go/No-Go odor discrimination task and found significant learning rate improvement for retronasally pre-exposed relative to novel odorants in a volatility dependent manner. The result suggests that retro- and orthonasal routes can generate similar perceptual qualities despite the differences between routes in odorant concentrations, airflow dynamics and orosensory activation. Thus, they may engage a similar neural mechanism for odor processing. In Experiment 2, rats were trained to discriminate tasteless retronasal odorized solutions, which allowed us to directly compare the breathing and licking behaviors as well as electrophysiological learning patterns between olfactory routes. Local field potential (LFP) recordings in the olfactory bulb, the olfactory tubercle and the piriform cortex show odor-evoked oscillation power in beta and gamma band for both ortho- and retronasal odor sampling. However, power spectral density and coherence patterns indicate different temporal patterns of oscillatory events and coherence structure, which appear to be modulated by differences in breathing and licking behaviors. We also examine the differences between system activity during licking in tasteless odor sampling and licking for the odorless taste reward solution; in this case the same motor behavior (licking) is involved in different perceptual modalities.

Disclosures: R. He: None. **K. Chen:** None. **L.M. Kay:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.25/L2

Topic: D.05. Olfaction and Taste

Support: NIH/NIDCD R01DC015784
NIH/NIDCD F31DC017661
NIH/NINDS R21NS104826
The Welch Foundation

Title: Sensitive and selective bile acid receptors in the vomeronasal organ

Authors: *W. WONG[1], J. CAO[1], X. ZHANG[1], W. I. DOYLE[2], J. P. MEEKS[1];
[1]UT Southwestern, Dallas, TX; [2]Cognitive Sci., UCSD, La Jolla, CA

Abstract: Terrestrial mammals rely heavily on the accessory olfactory system (AOS) for social and reproductive behaviors such as mating and territorial aggression. Previous studies revealed that fecal bile acids (BAs) are potent AOS activators. However, questions remain regarding the sensitivity and specificity of BA tuning. Moreover, it is unclear which of the approximately 300 sensory vomeronasal receptors (VRs) are responsible for BA sensitivity. To examine how the AOS detects and encodes BA information in the periphery, we measured BA tuning of thousands of VSNs using volumetric GCaMP6f/s Ca^{2+} imaging. We found that many VSNs displayed selective responsiveness to BAs, with some neurons responding to concentrations as low as 100 nM. We also observed sets of VSNs that displayed sensitive, but broad BA tuning. In addition, we stimulated VSNs with a broad panel of BAs and other polar steroids. We found that some VSNs were tuned to both BAs and sulfated steroids, most prominently urinary sulfated glucocorticoids, suggesting that many VSNs are tuned to steroids that span biological classes. Finally, to determine the receptors expressed by BA-sensitive VSNs, we developed a function-forward strategy to isolate highly BA-sensitive VSNs for single-cell RNA sequencing (scRNAseq). We identified BA-responsive VSNs in VNO slices via GCaMP6s imaging, then aspirated the whole soma and processed each cell for scRNAseq. We identified at least six V1R-family VRs. Collectively, these data reveal fundamental insights into the mechanisms of fecal BA sensation and improve our understanding of the organization of BA information as it flows through this behaviorally-relevant sensory system.

Disclosures: W. Wong: None. **J. Cao:** None. **X. Zhang:** None. **J.P. Meeks:** None. **W.I. Doyle:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.26/L3

Topic: D.05. Olfaction and Taste

Title: Kirrel3 and olfactory circuit formation and that contributes to neurodegenerative diseases in the mouse

Authors: *E. EERDUNFU, L. BAO, Y. WU, H. TAKEUCHI, Y. IKEGAYA;
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Abstract: In the mouse olfactory system, odorants are detected by ~1,000 different odorant receptors (ORs) which are produced by olfactory sensory neurons (OSNs) which are located in the olfactory epithelium (OE). Based on the "one neuron-one receptor" and "one glomerulus-one receptor" rules, binding signals of odorants detected by OSNs are converted to topographic information of activated glomeruli in the olfactory bulb (OB). During development, the glomerular map is formed by the combination of two genetically programmed processes: one is OR-independent projection along the dorsal-ventral axis, and the other is OR-dependent projection along the anterior-posterior axis. Odor information is transferred from the OB to the olfactory cortex (OC) through secondary olfactory neurons, which are called mitral cells. Mitral cells are produced in the ventricular zone (VZ) around E10-13 and migrate radially to the mitral cell layer through the intermediate zone in the OB. Each mitral cell extends a primary dendrite to single glomeruli and sends its axon branches to the OC. It has been known that single glomerulus connects with approximately 20-30 mitral cell dendrites. Recent electrophysiological study (Padmanabhanand Urban, 2010; Wilson, 2010) demonstrated that the mitral cell postsynaptic to the same glomeruli has diverse intrinsic properties. This result indicates that there must be genetic subtypes within the mitral cells connecting with the same glomeruli. Kirrel3 is a type 1 transmembrane protein and has been implicated in neuronal synapse development. *In situ* hybridization result revealed that Kirrel3 shows a punctuate expression in the mitral cell layer of the OB. Since Kirrel3 expression was not affected in the ΔD mouse, Kirrel3 expression presynaptic OSNs were genetically ablated by inducing diphtheria toxin, suggesting that Kirrel3 expression is regulated by intrinsic properties of mitral cells. Genetic labeling of Kirrel3-positive mitral cell axons revealed that kirrel3-positive axons project to part of the OC. Since Kirrel3 is a synaptic adhesion molecule, it is likely that kirrel3 protein controls the axonal projection of mitral cells. Result indicated that projection of Kirrel3 signals on the olfactory cortex occurs more diffused and broadly area compared with Kirrel3-positive and wild type mouse. It is looks like Kirrel3 as an important controller for the transduction of signal information process that was projected by mitral cells axon to the olfactory cortex. It is also reported that Kirrel3 is hidden risk gene in Alzheimer's disease.

Disclosures: **E. Eerdunfu:** None. **L. Bao:** None. **Y. Wu:** None. **H. Takeuchi:** None. **Y. Ikegaya:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.27/L4

Topic: D.05. Olfaction and Taste

Support: NIDCD Grant R00DC011780
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NSF Grant IOS-1451034
Welch Foundation Grant I-1934-20170325
the University of Texas System Neuroscience and Neurotechnology Institute

Title: The unique role of accessory olfactory bulb external granule cells in chemosensory information processing

Authors: *X. ZHANG[1], J. P. MEEKS[2];
[2]Neurosci., [1]UT Southwestern Med. Ctr., Dallas, TX

Abstract: Rodent social behavior relies heavily on the accessory olfactory system (AOS), an olfactory pathway best known for detecting pheromones. The accessory olfactory bulb (AOB) is the first and only dedicated circuit for AOS information processing, and information produced by the AOB powerfully influences social and reproductive behaviors. The local circuit in the AOB comprises projecting mitral cells (MCs) and several classes of local GABAergic interneurons. The role of inhibitory interneurons in sculpting information carried by MCs is mostly unclear. Here, we present Ca^{2+} imaging and electrophysiological data from ex *vivo* preparations of the AOS indicating how a novel population of AOB inhibitory interneurons, called external granule cells (EGCs), contribute to AOS information processing.
We performed two-photon GCaMP6f Ca^{2+} imaging of cortistatin-positive EGCs to evaluate their chemosensory tuning to a broad panel of monomolecular sulfated steroid ligands. EGCs, which previous experiments showed are extremely hyperpolarized at rest, responded very sparsely to monomolecular ligands at concentrations that robustly activate MCs. However, EGCs robustly responded to natural pheromone-containing substances (dilute mouse urine and fecal extracts). Compared to AOB MCs, the sparse responses of EGC indicate that these cells are only engaged during conditions in which many individual ligands are simultaneously encountered. *Ex vivo* targeted whole cell patch clamp recordings revealed broad monomolecular-ligand-evoked subthreshold activity in EGCs, confirming that these cells are broadly innervated by MCs. In sum, our data indicate that AOB EGCs are broadly-integrating cells that possess extremely high

activation thresholds. The unique physiological features of AOB EGCs distinguish them from analogous cells in the main olfactory bulb, and indicate how they contribute to the processing of real-world chemosignals.

Disclosures: X. Zhang: None. **J.P. Meeks:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.28/L5

Topic: D.05. Olfaction and Taste

Support: NIH DC012567
Firmenich

Title: Modulation of primary olfactory receptor activity by mixtures

Authors: *L. XU[1], W. LI[2], V. VOLETI[2], E. M. HILLMAN[3], S. J. FIRESTEIN[4];
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Abstract: Olfactory receptors are the largest family of Class A GPCRs, outnumbering all the other identified receptors by more than 2:1. Therefore they are useful candidates for studying function in GPCRs as well as transduction of olfactory sensory signals. Most olfactory stimuli are blends of odors consisting of between a two or three and hundreds of molecular components. Therefore most stimuli likely activate multiple receptors and the pattern of receptor activity is thought to be a signature for that stimulus. Taking advantage of a novel microscopy technique, Swept Confocally Aligned Planar Excitation (SCAPE), we have been able to record the simultaneous response to mixtures of odors of over 10,000 olfactory sensory neurons in the olfactory epithelium of a single mouse. In these recordings we find evidence for extensive modulation of receptor activity by different component molecules. Not only do molecules activate receptors (agonists) but they may also act at other receptors as antagonists. In addition we have also observed many cases of apparent non-linear enhancement of some responses by mixture components suggesting the possibility of allosteric modulation, previously unknown in Class A GPCRs.

Disclosures: L. Xu: None. **W. Li:** None. **V. Voleti:** None. **E.M. Hillman:** None. **S.J. Firestein:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.29/L6

Topic: D.05. Olfaction and Taste

Support: NIH NIDCD R01 DC016222

Title: Snowballs or snowflakes: Transcriptional heterogeneity in olfactory sensory neurons

Authors: *T. TSUKAHARA, D. H. BRANN, S. R. DATTA;
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Abstract: Odor perception is essential for animals to execute appropriate behaviors in the complex natural environment. To faithfully represent the external chemical information, olfaction relies on the heterogeneity in olfactory sensory neurons (OSNs) created by the Olfactory Receptors (ORs). In mammals each OSN expresses only one OR out of a large repertoire (more than 1000 in mice); however, all ORs use a common signaling pathway downstream of ligand-binding. This suggests a model in which all OSNs are equivalent to each other and are distinguished only by ORs they express. On the other hand, the olfactory epithelium is divided into multiple anatomical zones. Cells expressing a given OR are spatially restricted to one of such zones and project axons to a single pair of glomeruli in the olfactory bulb. While ORs are known to regulate a small number of axon guidance genes to enable this convergent axon targeting during early postnatal days, it remains unclear to what extent the OSNs that express each OR are unique, especially in adult animals. We used single cell RNA-sequencing to comprehensively understand the molecular heterogeneity in OSNs. We successfully identified all the known cell types in the main olfactory epithelium. In mature OSNs, we detected almost all the OR genes with different frequencies and did not find any evidence that clearly contradicts to one-receptor-per-neuron pattern. We also identified known subtypes of OSNs such as dorsal, ventral and recently identified CD36-positive neurons, but we did not identify any novel distinct clusters of OSNs, supporting a model in which all OSNs are more or less generic. However, we were also able to identify sets of genes whose expression varied in orthogonal ways across OSNs. Current experiments are underway to identify the sources and functional consequences of this variation and heterogeneity across OSNs.

Disclosures: T. Tsukahara: None. **D.H. Brann:** None. **S.R. Datta:** None.

Poster

058. Chemosensory Processing I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 058.30/L7

Topic: D.05. Olfaction and Taste

Title: Widespread inhibitory responses in the mouse olfactory sensory neurons *in vivo*

Authors: *S. INAGAKI, R. IWATA, T. IMAI;
Dept. of Developmental Neurophysiol., Kyushu Univ., Fukuoka, Japan

Abstract: It is generally believed that odorants "activate" odorant receptors (ORs) and odor information is spatiotemporally represented by the "activation" patterns of glomeruli in the olfactory bulb (OB). Here we performed in vivo two-photon Ca^{2+} imaging of OSN axon terminals in the OB, and found that over 5% of glomeruli show robust inhibitory responses to odors, while ~25% demonstrated excitation. Temporal kinetics of the inhibitory responses was typically slower than that of excitatory ones. As OSNs are known to show spontaneous activity without odors, this is most likely due to the reduction of spontaneous activity in OSNs. To examine a possible role for interglomerular presynaptic inhibition by GABA and dopamine, we generated an OSN-specific knockout of $GABA_B$ receptors and dopamine D2 receptors. However, inhibitory responses were still seen at OSN axon terminals in ~3% of glomeruli. To examine whether unknown forms of pre-synaptic inhibition are involved, we also generated OSN-specific tetanus toxin light chain (TeNT) transgenic animals, in which synaptic transmission from all OSNs was blocked; however, we still observed robust inhibitory responses in ONS axons, suggesting that at least a fraction of the inhibition occurs non-synaptically. We therefore performed two-photon Ca^{2+} imaging of OSN somata in the olfactory epithelium (OE) *in vivo*, and found that the inhibitory responses are already happening at the OSN somata. In *Drosophila*, reduction of basal OR current and non-synaptic lateral inhibition via ephaptic coupling are known to produce inhibitory responses in some OSNs. Therefore, similar mechanisms may underlie the widespread inhibition in the mammalian OSNs. Similarly to the visual system, both excitation and inhibition of OSNs may contribute to the efficient olfactory coding.

Disclosures: **S. Inagaki:** None. **R. Iwata:** None. **T. Imai:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.01/L8

Topic: D.06. Auditory & Vestibular Systems

Support: ERC Consolidator Grant AUDADAPT 646696 (

Title: Tracking of 1/f stimulus characteristics in the human electroencephalogram

Authors: *L. WASCHKE[1], T. DONOGHUE[2], S. SMITH[2], B. VOYTEK[2], J. OBLESER[1]; [1]Dept. Of Psychology, Univ. of Luebeck, Luebeck, Germany; [2]Univ. of California San Diego Dept. of Cognitive Sci., La Jolla, CA

Abstract: The statistics of both human electrophysiology data and many natural stimuli follow a power law, that is, they share a $1/f^{\chi}$ distribution. Both kinds of time series hence are characterized by pronounced events that occur with relatively low frequency compared to more subtle fluctuations arising with higher frequency. This reduction in signal magnitude with increasing frequency is aptly captured by the slope, or exponent (χ) of the power spectral density (PSD).

Recently, the spectral exponent of electrophysiological recordings has been suggested as a marker of inter-individual traits and behaviourally relevant brain states. Specifically, the spectral exponent captures aperiodic, non-oscillatory parts of electrophysiological signals and has been hypothesized to reflect the balance of excitatory and inhibitory activity (E:I ratio) in populations of cortical neurons. It is unclear, however, to which degree bottom-up influences like stimulus characteristics alter the electrophysiological spectral exponent. Additionally, the impact of top-down processes such as the selective allocation of cognitive resources on the PSD and its interplay with sensory factors is unknown.

We here present evidence from two different experiments, during which we recorded electroencephalography (EEG) while participants (total N = 44) detected faint target stimuli in streams of auditory or visual noise. Importantly, all noise stimuli were generated to exhibit different $1/f\ \chi$ values ($1/f^0$, $1/f^1$, $1/f^2$, $1/f^3$) in their modulation spectra.

Spectral exponents over auditory and visual sensory cortices were positively related to χ values of the respective stimulus domain, and thus tracked stimulus characteristics on the single-trial level. Notably, conventional metrics of EEG analysis such as event related potentials were insensitive to these changes. The tracking of stimulus statistics did not hinge on modality-specific attention. However, we find that modality-specific attention reduced spectral exponents, i.e. caused a flattening of EEG spectra. Within the E:I framework, this would related to an increased E:I ratio (E>I) over sensory regions of the attended domain.

These results demonstrate the importance of aperiodic, non-oscillatory component of

electrophysiological signals, captured by the spectral exponent, for the study of sensory and cognitive functions. We relate our results with the tracking of stimulus information in the time domain using temporal response functions and discuss the relevance of tracking stimulus statistics for behaviour.

Disclosures: L. Waschke: None. **T. Donoghue:** None. **S. Smith:** None. **B. Voytek:** None. **J. Obleser:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.02/L9

Topic: D.06. Auditory & Vestibular Systems

Support: The Hoffman fellowship to TIR
Jack H. Skirball research fund to LYD
The Israel Academy of Sciences (390/ 13) to IN

Title: Multiple timescale sensitivity of EEG components to statistical features in unattended tone sequences

Authors: *T. I. REGEV[1], G. MARKUSFELD[2], I. NELKEN[1,3], L. Y. DEOUELL[1,2];
[1]Edmond and Lily Safra Ctr. for Brain Sci., [2]Psychology Dept., [3]Neurobio. Dept., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Everyday auditory stimuli, such as in music and speech, contain relevant statistical features in multiple timescales. We demonstrate sensitivity of ERP components to the distribution of auditory frequencies in an unattended tone sequence. In three EEG experiments, 82 participants (21, 28 and 33 in Experiments 1, 2 and 3, respectively) were instructed to ignore tone sequences while viewing a silent film. The sequences were comprised of five equiprobable tones. The tones' frequencies were distributed across four octaves in Experiments 1 and 2, while in Experiment 3 this range varied between large, medium, and small (4, 2 or 1 octaves, respectively). We found that the amplitude of the N1 component - a negative EEG deflection peaking about 100 milliseconds after tone onset - was sensitive to the absolute log distance between the current tone's frequency and the mean frequency of the tones in the sequence: The farther the tone's frequency from the mean, the larger the evoked N1 amplitude. In contrast, the P2 component - a positive deflection peaking about 200 milliseconds after tone onset - showed a temporally local sensitivity to the log interval between the current and previous tones' frequencies, and a weaker sensitivity to the sequence mean frequency. These results were replicated across the 3 experiments. We propose a simple biophysical model of adapting neurons with wide frequency tuning curves and multiple adaptation time constants to explain these

results. Our results give electrophysiological evidence for pre-attentive simultaneous monitoring of distributions of sound features at multiple timescales in the human auditory cortex.

Disclosures: **T.I. Regev:** None. **G. Markusfeld:** None. **I. Nelken:** None. **L.Y. Deouell:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.03/L10

Topic: D.06. Auditory & Vestibular Systems

Support: JSPS KAKENHI 17H01769
JSPS KAKENHI 18J21644
JSPS KAKENHI 18H05089

Title: Cortical activity induced by laser stimulation of auditory nerves

Authors: ***Y. TAMAI**[1], Y. ITO[2], T. FURUYAMA[1], K. HORINOUCHI[1], N. MURASHIMA[1], I. MICHIMOTO[1], S. HIRYU[1], K. I. KOBAYASHI[1];
[2]Grad. Sch. of Life and Med. Sci., [1]Doshisha Univ., Kyoto, Japan

Abstract: Sensorineural hearing loss is characterized by malfunction in the translation of sound information into neural electrical activity. A cochlear implant allows a hearing impaired individuals to regain a auditory sense by electrically stimulating the cochlear nerves, bypassing damaged hair cells. However, the devices also have problems of invasiveness of surgical intervention. In the present study, we proposed application of infrared neural stimulation to a hearing aid because pulsed infrared laser elicits neural activity without contacting tissues. The purpose of this study was to quantifying cortical activity elicited by laser stimulation of auditory nerve. Flavoprotein fluorescence imaging of left hemisphere of the brain was performed to assess the cortical response. Mongolian gerbil (*Meriones unguiculatus*) were anesthetized with urethane. Cortical images of endogenous green fluorescence in blue light were captured by camera through the intact skull. An optic fiber was inserted into a subject's ear canal without contacting a tympanic membrane, and lateral side of second turn of subject's cochlea was irradiated with infrared laser for stimulating auditory nerves. Visual (red LED light), tactile (whisker vibration), and auditory stimuli (noise burst; frequency: 1-52 kHz) were presented for locating visual, somatosensory, and auditory cortex. Pulsed infrared laser (repetition rate: 4 kHz) were used as laser stimulus, and their radiant exposure was adjusted to 0.7-12.8 mJ/cm^2 per pulse. As results, laser stimulation activated relatively broad area of auditory cortex as noise burst did. Response amplitude and latency elicited by laser stimulation was significantly correlated with radiant exposure (amplitude: $r=0.54$, $p<0.05$; latency: $r=-0.49$, $p<0.05$), and these variations were comparable to the auditory responses. The results suggest that laser-induced

cochlear nerve response is processed in auditory cortex, and radiant energy can be one of the optimal parameters for manipulating auditory cortical activities.

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Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.04/L11

Topic: D.06. Auditory & Vestibular Systems

Support: NICHD Grant RO1-HD29419 to AAB
Elizabeth H. Solomon Center for Neurodevelopmental Research

Title: Variations in oscillatory patterns of spectral power and phase coherence may underlie deficits in rapid auditory processing for 12-month-old infants at familial risk for language learning impairment

Authors: *S. ORTIZ-MANTILLA, T. REALPE-BONILLA, C. P. ROESLER, A. A. BENASICH;
Rutgers Univ. Newark, Newark, NJ

Abstract: The ability to perform fine-grained acoustic analysis in the tens of milliseconds range, known as Rapid Auditory Processing (RAP), is essential to decoding information in the speech stream and critical in infancy to establish accurate cortical phonemic representations. Infants born to a family with a history of language learning impairment (LLI) are known to be at high-risk for developing LLI. Behavioral, electrophysiological and genetic studies suggest that in these high-risk infants RAP abilities are affected. However, little is known about the oscillatory mechanisms supporting RAP abilities in typical and at-risk infant development. In this study, 12-month-old infants at high-risk for LLI because of family history (FH) were presented a passive oddball paradigm with fast-rate tone-pairs containing a frequency contrast and then compared to 12-month-old typical control infants (CT). Event-related cortical signals were mapped onto infant brain templates. Dipole-source modeling placed generators for each group's event-related responses to standard (ST) and deviant (DV) stimuli in left (LAC) and right (RAC) auditory cortices. Time frequency analysis was conducted in source space within the 2 to 90 Hz frequency range. Differences in spectral power and inter-trial phase coherence were examined via permutation testing and cluster analysis. Lateralization differences in high frequency oscillatory patterns (gamma) were seen between the groups. For spectral power, the CT group showed increased left gamma power for the DV, whereas the FH group showed increased left beta-gamma power for the ST. For inter-trial phase coherence, enhancement of phase coherence in the

gamma range was seen for the DV in both CT and FH groups. Furthermore, while the CT group showed greater bilateral phase coherence for the DV in the 54-60 Hz range, the FH group showed this increase only in LAC and in a lower gamma frequency range (40-51 Hz). In addition, the CT group exhibited more theta phase coherence in RAC than the FH group for both DV and ST. Differences seen between the groups in the amount and laterality of spectral power and inter-trial phase coherence during RAP suggest oscillatory dynamics in FH infants may be disrupted. The left auditory cortex has often been related to rapid temporal processing. Fast-rate gamma oscillations are particularly suited for processing acoustic information that varies in the tens of milliseconds range. We suggest that the reduced amounts of left gamma power elicited by the FH group during deviant discrimination may be one of the underlying mechanisms supporting the aberrant processing observed in infants at risk for LLI during RAP.

Disclosures: S. Ortiz-Mantilla: None. **T. Realpe-Bonilla:** None. **C.P. Roesler:** None. **A.A. Benasich:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.05/L12

Topic: D.06. Auditory & Vestibular Systems

Support: EC/RGC HK E_Cityu101/17

Title: Sequence learning in mammals: An animal model to explore the mechanisms of language acquisition

Authors: *D. LUO[1], R. AUKSZTULEWICZ[1,2], J. W. SCHNUPP[1];
[1]Dept. of Biomed. Sci., City Univ. of Hong Kong, Hong Kong, Hong Kong; [2]Dept. of Neurosci., Max Planck Inst. for Empirical Aesthetics, Frankfurt, Germany

Abstract: The ability to segment a stimulus sequence in a continuous auditory stream into separate chunks is critical to humans and other animals. In humans, sequence learning ability is likely one of the building blocks of language acquisition, and infants are capable to detect novel stimuli in a continuous speech stream after passive exposure to familiar stimuli [1]. Non-invasive studies in humans suggest that low-frequency cortical entrainment to the rate of presentation of speech segments is modulated by language familiarity [2]. Interestingly, also primates [3] and rodents [4] have been suggested to be able to chunk acoustic streams. Here, we test whether low-frequency entrainment mediates auditory chunking in rodents.
In this study, we trained female Wistar rats (N=7) to perform sequence familiarity discrimination of synthetized acoustic syllable triplets in two-alternative forced choice tasks. First, rats were trained to differentiate 3 single repeatedly presented syllables (‘familiar’) from their scrambled

counterparts, generated anew in each trial ('unfamiliar'). Then, single syllables were replaced with 3 triplets, and rats were trained to discriminate familiar triplets from unfamiliar triplets. Finally, rats were anaesthetized and implanted with electrocorticographic electrode covering their auditory and frontal regions. Rats in a control group (N=7) were passively exposed to familiar stimuli. During electrophysiology experiments, rats were presented with continuous streams of familiar triplets as well as streams containing unfamiliar stimuli. We tested (1) whether cortical activity could entrain to the triplet presentation rate (showing a difference between familiar and unfamiliar triplets), and (2) whether this effect was modulated by active vs. passive training.
Our behavioral data show that rats were able to discriminate familiar syllables and triplets from the reshuffled stimuli. In the electrophysiology data we observed a robust difference in cortical responses to familiar vs. unfamiliar triplets in actively trained rats. For passively exposed rats, the effect of triplet familiarity on cortical activity was diminished. These results suggest that rats can chunk continuous stimulus streams into reproducible segments following active training.

Reference

[1] Saffran JR, Aslin RN, Newport EL (1996). *Science 274*(5294):1926–1928.
[2] Ding N, Melloni L, Zhang H, Tian X, Poeppel D (2015) *Nature Neuroscience 19*(1):158–164.
[3] Hauser, M. D., & Fitch, W. T. (2004). Computational constraints on syntactic processing in a non-human. *Science*, *303*, 377–380.
[4] Murphy RA, Mondragon E, Murphy VA (2008) *Science 319*(5871):1849–1851.

Disclosures: D. Luo: None. **R. Auksztulewicz:** None. **J.W. Schnupp:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.06/L13

Topic: D.06. Auditory & Vestibular Systems

Support: JSPS KAKENHI JP25351001

Title: Memory-based and cross-modal acceleration effects on auditory steady-state response

Authors: *S. SUGIYAMA[1], M. NISHIHARA[2], K. INUI[3];
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Abstract: Steady state responses (SSRs) are electrophysiological responses driven by a train of stimuli delivered at a high enough rate. The effect of a salient sensory stimulus on a SSR is known as phase resetting, and it induces modulation of the amplitude and phase of the SSR.

Because auditory steady state response (ASSR) is known to reach maximum amplitude near 40 Hz, many studies have reported on phase resetting of 40 Hz-ASSR, revealing that ASSR was modulated by stimulus onset, changes in periodicity of the sound stimulus, and the presence of an interfering stimulus. In the present study, we performed two experiments using magnetoencephalography to investigate whether phase resetting of ASSR—especially its temporal aspect—was influenced by the probability of the perturbing sound stimulus under an oddball paradigm (Experiment 1), and was influenced by the tactile stimulation (Experiment 2). This study was approved in advance by the Ethics Committee of the National Institute for Physiological Sciences, Okazaki, Japan, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. **Experiment 1**: Twelve healthy human subjects completed an oddball paradigm with a sudden sound pressure change as the test stimulus, i.e., the control stimulus was a train of 25-ms pure tones at 75 dB for 1200 ms, while the 29th sound at 700 ms of the test stimulus was replaced with a 90-dB tone. Then, we compared the latency of ASSR among four probabilities of test stimulus (0, 25, 75, and 100%). For both the control and test stimulus, stronger effects of acceleration on ASSR were observed when the stimulus was rarer. **Experiment 2**: A 0.5-ms electrical pulse was randomly presented to the dorsum of left or right hand of twelve healthy volunteers at 700 ms when a train of 25-ms pure tones were applied to the left or right side at 75 dB for 1200 ms. The peak latencies of ASSR compared across the conditions. The tactile stimulation significantly shortened the subsequent ASSR latency. These findings indicated that ASSR depended on physical changes as well as sensory memory and comparison processes, ASSR was modulated without peripheral inputs, and brain areas higher than the primary cortex were involved in exerting acceleration effects. The cross-modal effect of tactile inputs on ASSR was also confirmed for the first time. The reduced ASSR latency clearly indicated that a new sensory event increased the speed of ongoing sensory processing. Thus, changes in ASSR latency are a sensitive index of accelerated processing.

Disclosures: **S. Sugiyama:** None. **M. Nishihara:** None. **K. Inui:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.07/L14

Topic: D.06. Auditory & Vestibular Systems

Support: FIG grant Lehigh university

Title: The development of tonotopic specialization of the chick cochlear nucleus

Authors: *L. S. JONES[1], R. BURGER[1], M. KELLEY[2], Z. MANN[3];
[1]Lehigh Univ., Bethlehem, PA; [2]NIDCD, NIH, Bethesda, MD; [3]Kings Col. London, London, United Kingdom

Abstract: Tonotopic organization is the fundamental organizing principle of the auditory system. In the avian cochlea this pattern arises along the basilar papilla and is defined partially by hair cell morphological and physiological gradients from base to apex. The BP imparts tonotopic organization on to central auditory neurons via the 8th nerve which provides primary input to Nucleus magnocellularis (NM). A primary function of NM neurons is to preserve or improve temporal precision of phase-locked discharges to sounds, a key physiological feature for sound localization. These neurons are able to accomplish this task at relatively high frequencies due to tonotopically distributed intrinsic membrane properties. These include distributed expression of voltage-gated ion channels along the tonotopic gradient. Additionally, 8th nerve synapse number and size varies similarly along this gradient.
One unresolved question is how tonotopic properties in the brain arise during development? One hypothesis is that tonotopically distributed properties first develop in the ear which then, in turn, drives the development of intrinsic properties centrally. An alternative, but not mutually exclusive, hypothesis is that central neurons develop independently of the ear instead relying on central cues to establish tonotopic patterns. We investigated this question using a "monotopic" chick model. Previous work by the Kelley lab (Mann et al, 2014) showed that a gradient of Bone morphogenic protein 7 (BMP7) is a primary driver of tonotopic patterning of hair cells along the BP. Overexpression of BMP7 in the developing otocyst results in a shift toward low frequency-like phenotypes along the entire BP.
We overexpressed BMP7 in-ovo using an RCAS viral vector to create chicks with a low-frequency like ear. We examined the development of intrinsic cellular properties within these animals. To investigate NM neuron excitability along the tonotopic axis, we used whole-cell current clamp to inject ramp stimuli into NM neurons. This enabled the measurement of slope threshold (ST) and integration period (IP) values, which are known to differ significantly among high and low characteristic frequency NM neurons (HCF, LCF) (Oline et al, 2016). Our results suggest that ST and IP value distributions across NM shift toward values expressed in control low characteristic frequency neurons. These data are the first to suggest that intrinsic membrane properties of central neurons are driven by the organizational features of the sensory epithelium.

Disclosures: L.S. Jones: None. **R. Burger:** None. **M. Kelley:** None. **Z. Mann:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.08/L15

Topic: D.06. Auditory & Vestibular Systems

Support: Cambrdige Trust
Trinity Henry-Barlow Scholarship

Title: Neural circuit and processing mechanism underlying auditory pattern recognition in the cricket brain

Authors: *X. ZHANG, B. HEDWIG;
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Abstract: Recognition of species-specific sound signals is fundamental to all acoustically communicating animals. However, the neural mechanisms that process and compute the temporal structure of a sound signal are poorly understood. Studying the phonotactic behaviour of crickets, which is driven by simple pulse and chirp patterns, has advanced our understanding of pattern recognition in the central nervous system.
As the neuronal basis of song pattern recognition, a delay-line and coincidence-detector circuit has been revealed in the brain of female crickets. It is composed of the axonal output structures of an ascending auditory interneuron and 4 local neurons. Within this circuit, a non-spiking interneuron, which is inhibited in response to a sound pulse, functions as a delay-line and forwards a delayed post-inhibitory rebound to the coincidence detector neuron. The coincidence detector neuron responds strongest when the delay of the input via the non-spiking neuron corresponds to the pulse period of the song. This can be explained by the coincidence of the delayed and the direct inputs. As a consequence, the feature detector will be activated and thus establishes the basis for species-specific pulse pattern recognition.
The functional properties of the circuit are shaped by the sequential rather than discrete processing of individual sound pulses. Different behavioural responses result from varying the temporal structure of sound pulses in a chirp have led to a deeper insight into the processing mechanism, e.g. the animals tolerate long pulses at the end of a chirp but not at the beginning. The neuronal activities in response to these sound stimuli were also analyzed to unravel how the dynamics of membrane potential determines this irreversible processing. In particular, based on the behavioural result we generated attractive and non-attractive chirp patterns, in which the sound pulses are the same but played in reverse order. This discrepancy is also reflected at the neuronal level. For example, the final feature detector neuron of the circuit responds with two significant bursts of spikes to the attractive pattern whereas only one burst is generated in response to the non-attractive pattern.
Additional recordings of brain neurons (n>500) has not only shown the contribution of the delay-line and coincidence-detector on processing the inter-pulse intervals but also indicated a pulse duration filtering circuit. Theses circuits form a computational network for coding and recognizing the specific temporal pattern of species-specific auditory signals.

Disclosures: **X. Zhang:** None. **B. Hedwig:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.09/L16

Topic: D.06. Auditory & Vestibular Systems

Support: ERC Consolidator award (SOUNDSCENE)
Wellcome Trust / Royal Society fellowship to JKB (098418/Z/12/Z)

Title: Behavioural and neural measures of auditory regularity detection in ferrets

Authors: *K. C. POOLE, E. J. JONES, J. K. BIZLEY;
UCL Ear Inst., Univ. Col. London, London, United Kingdom

Abstract: To understand complex acoustic environments humans and animals are able to segregate and maintain auditory streams during auditory scene analysis (ASA). The brain can perform efficient scene analysis by using stimulus statistics to extract regularities and predict future events, utilising ongoing context and temporal structure. As a result, acoustic stimuli that transition from random to regular pure tone sequences have recently been employed as an objective measure to investigate ASA (Barascud et al., 2016).
We trained ferrets (n=3) on a go/no-go task where the animals are required to detect the transition from a random sequence of tones (50ms duration) to a regularly repeating sequence (repeat length 3 tones, duration 150ms), and simultaneously in a visual task where a continuously lit LED transitions to a flashing LED (flash interval 150ms). All animals were able to perform significantly above chance (Monte-Carlo simulation) in each modality (Auditory: $p < 0.001$, d' range across animals = 1.02 to 1.70; Visual: $p < 0.001$, d' range across animals = 1.7 to 2.03). To test increasingly complex auditory patterns, repeat lengths were varied from 3 to 5, 7 and 10 tones with performance significantly above chance for all repeat lengths ($p < 0.001$, respective mean d' across animals: 1.25, 1.35, 1.81, 0.82).
With these data paving the way for investigating regularity detection, we aim to identify the neural correlates of this auditory process by recording local field potentials and spiking activity in the auditory cortex of trained animals engaged in this behavioural task, with comparison to neural activity in passive naïve animals.

References

Barascud, N., Pearce, M.T., Griffiths, T.D., Friston, K.J., and Chait, M. (2016). Brain responses in humans reveal ideal observer-like sensitivity to complex acoustic patterns. PNAS 113, E616-E625.

Disclosures: K.C. Poole: None. **E.J. Jones:** None. **J.K. Bizley:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.10/L17

Topic: D.06. Auditory & Vestibular Systems

Support: NIH (NIDCD Grant DCO2514)

Title: Effects of stochastically varying modulation frequency on the detection of amplitude-modulated noise

Authors: *K. N. O'CONNOR[1], D. R. JOHNSON[2], J. S. JOHNSON[2], M. L. SUTTER[1];
[1]Ctr. for Neurosci. and Dept. of Neurobiology, Physiol. and Behavior, [2]Ctr. for Neurosci., UC Davis, Davis, CA

Abstract: Amplitude modulated (AM) noise has been a useful tool for investigating temporal processing in auditory systems because no confounding spectral information is present. The vast bulk of research has been performed using sine-AM (SAM), which allows determination of AM sensitivity as a function of modulation frequency. Analysis of neural responses to SAM noise has produced two primary coding models, one based on spike rate and the other on the temporal pattern of spikes (Joris et. al. 2004), which for SAM, is roughly phase-locked to the oscillations in the signal.

One problem for this temporal coding scheme is the limitation in neurons' ability to follow fast oscillations as the auditory system is ascended. For this reason, it has been proposed that, for auditory cortex (AC), a rate code is used for high AM frequencies, while temporal coding occurs at lower frequencies (< ~60 Hz) where synchronous responding is prevalent (Wang 2008), though this is not a clear-cut distinction (Yin et. al. 2011; Johnson et. al. 2012). In macaque AC firing rate is more closely linked to behavioral AM detection than synchronicity, implying a greater role for firing rate in perception and decision making (Niwa et.al. 2012; 2013). To test for the role of synchronous following in encoding AM, we introduced stimuli whose modulation frequency varied stochastically about a center frequency (CMF) and across several modulation frequency ranges or bandwidths (MBWs). A simple phase-locking code should degrade as the variation about the CMF increases. The ability of 3 listeners (3 adult males) to detect modulation in these stochastically modulated AM (400-ms) noise stimuli, as a function of modulation level, was tested. Modulation detection was tested at 2 CMFs (20 and 250 Hz) and at 3 MBWs (1, 2 and 4 octaves), and also for SAM noise at the CMFs. If synchronous phase-locked responding is important for detecting AM noise, detection should worsen with increasing MBW, and to a much greater degree for the 20 than 250 Hz CMF stimuli. We did find poorer detection with increasing MBW at both CMFs. Though there was no interaction between CMF and MBW, detection at 2 and 4 octave MBWs was notably worse for 20 Hz CMF stimuli. The results indicate that,

although synchronous oscillatory neural activity may be a useful cortical temporal code at low frequencies, it is not essential.

Disclosures: K.N. O'Connor: None. **D.R. Johnson:** None. **J.S. Johnson:** None. **M.L. Sutter:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.11/L18

Topic: D.06. Auditory & Vestibular Systems

Support: Czech Science Foundation Grant 18-09692S

Title: Age related changes in peripheral and central auditory processing in Fischer 344 rats

Authors: *D. SUTA[1,2], K. PYSANENKO[1], J. POPELAR[1], Z. BURES[1], N. RYBALKO[1], T.-W. CHIU[3], J. SYKA[1,2];
[1]Inst. of Exptl. Med. CAS, Prague, Czech Republic; [2]CIIRC Czech Tech. Univ., Prague, Czech Republic; [3]Dept of Biol. Sci. and Technology, NCTU, Hsinchu, Taiwan

Abstract: Age-related decline of the auditory function is typically accompanied by functional changes of the peripheral as well central auditory processing. We compared rat´s hearing function and auditory temporal processing at peripheral and central levels in adult (6-11 months) and aged (23-32 months) rats of the fast aging rat strain Fischer 344 (F344) by recording of auditory brainstem responses (ABRs) and cortical middle latency responses (MLRs, recorded from the auditory cortex surface). In aged animals, their hearing thresholds for tones were elevated in the whole tested frequency range 2-40 kHz by 20-30 dB when compared to those obtained in adult rats. The thresholds to clicks were higher in aged animals by ~20 dB. Temporal processing of acoustical signals was investigated using acoustical stimulation with a pair of clicks and a gap-in-noise; both types of stimulations were used either in quiet or in background noise (i.e. clics in noise or gap partly filled by noise). The ABR as well as MLR amplitudes to both types of stimuli - clicks and gap - were significantly smaller in aged rats in comparison with adult rats. ABR as well as MLR amplitudes to click stimulation decreased with increasing intensity of the background noise. The ABR to the second click reached amplitudes similar to the first click at the inter-click interval (ICI) as short as 3 ms in both adult and aged rats. In case of MLR, amplitudes to the second click gradually increased for ICIs 30-200 ms. This increase of the MLR amplitude to the second click (when measured relatively to the first click) was not dependent on the background noise intensity in adult rats, but was impaired by background noise in aged rats. ABR responses to gap demonstrated worse temporal resolution in aged animals with thresholds ~10 ms, while adult animals responded to much shorter gaps ~3 ms. The presence of

an additional noise partially filling the gap resulted in a suppression of the amplitude of ABR response, the MLR amplitude was less affected. Our results demonstrate pronounced age-related changes in the auditory temporal processing particularly in the presence of the background noise.

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Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.12/L19

Topic: D.06. Auditory & Vestibular Systems

Support: Del Monte Institute of Neuroscience

Title: Including measures of high gamma power can improve the decoding of natural speech from EEG

Authors: *S. R. SYNIGAL[1], E. S. TEOH[3], E. C. LALOR[1,2,3];
[1]Biomed. Engin., [2]Neurosci. and Del Monte Inst. for Neurosci., Univ. of Rochester, Rochester, NY; [3]Sch. of Engineering, Trinity Ctr. for Biomed. Engineering, Trinity Col. Inst. of Neurosc., Trinity Col. Dublin, Univ. of Dublin, Dublin, Ireland

Abstract: The human auditory system is capable of extracting relevant features from speech with high fidelity, and electroencephalography (EEG) can serve as a non-invasive tool to examine this process. The brain has been shown to track the temporal envelope of speech, with the phase of low frequencies below 20 Hz being often associated with intelligibility. Electrocorticography studies have shown that high gamma power (HGP) activity also tracks the dynamics of speech, yet these signals are often discarded in EEG studies due to the skull's low pass filtering characteristics. The purpose of this study was to determine if HGP EEG carries useful stimulus-related information, and if so, might that information be complementary to that carried by low-frequency (LF) data. Specifically, we used linear regression to investigate speech envelope encoding and attention detection using LF, HGP, and combined LF+HGP neural signals. EEG data was collected while (1) 17 healthy subjects (12 males) listened to continuous natural speech and (2) while 14 other healthy subjects (6 male) attended to one of two concurrent speakers ('cocktail party'). HGP EEG decoded the speech envelope better than chance both within and across subjects. Decoders trained on LF and LF+HGP were able to successfully reconstruct the envelope of the heard speech as well. These decoders performed similarly across subjects and performed better than decoders trained on HGP alone. However, for several individual subjects, decoding was improved when using LF+HGP EEG compared to LF alone. Analysis of the relative contributions of each EEG channel to decoding showed that LF EEG at

frontotemporal electrodes were most important for envelope reconstruction, whereas HGP EEG at occipitotemporal electrodes were most important. With regards to the cocktail party task, we found that HGP could be used to ascertain attentional selection above chance but was less accurate than LF on a group level. Nevertheless, for several subjects, combining LF+HGP resulted in improved decoding accuracy than using LF alone. Overall, our results show that HGP EEG carries useful information regarding speech processing and attentional selection, and for some subjects this information is complementary to LF EEG. For those subjects, combining LF and HGP neural signals can improve the mapping between natural speech and the resulting neural responses.

Disclosures: **S.R. Synigal:** None. **E.S. Teoh:** None. **E.C. Lalor:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

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Program #/Poster #: 059.13/L20

Topic: D.06. Auditory & Vestibular Systems

Support: NIH 1UL1TR000460
NIH 1R21DC014324
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Wallace H Coulter Center for Translational Research
Cochlear Research Grant

Title: Mild therapeutic hypothermia preserves residual hearing against cochlear implant trauma: Preclinical results and translational potential

Authors: *R. SANGALETTI[1], E. A. DUGAN[2], C. KING[6], D. DIETRICH[3], M. HOFFER[4], S. RAJGURU[5];
[1]Otolaryngology, [2]Biomed. Engin., [3]Dept. of Neurolog. Surgery and Biomed. Engin., [4]Otolaryngology and Neurolog. Surgery, Univ. of Miami, Miami, FL; [5]Biomed. Engin. and Otolaryngology, Univ. of Miami, Coral Gables, FL; [6]Lucent Med. Systems, Seattle, WA

Abstract: More than half a million patients, including children, have benefitted significantly from the remarkable technological breakthrough that are cochlear implants (CI). An increasing number of cochlear implant subjects have some level of residual hearing at the time of implantation and can benefit from bimodal electro-acoustic devices. Surviving hair cell activity and as a result a functioning organ of Corti and neural substrate has recently been linked to speech perception outcomes. However, trauma to the sensitive inner ear structures during CI leads to inflammation and oxidative stress exacerbating the loss of residual hearing. A successful translation of therapeutic interventions to limit this trauma and protect residual hearing have yet

to be achieved. Here, we will review efforts to develop an application of localized, mild therapeutic hypothermia for protection of hair cells and the neural substrate following CI. We have developed a custom-probe and system to locally deliver mild hypothermia to the inner ear during CI surgery and preclinical results in a rodent model show significant improvement in survival of hair cells and preservation of residual function post-CI. We show that therapeutic hypothermia delivered to the inner ear is safe and does not adversely affect function. We used RNA-sequencing (RNA-Seq) and real-time PCR (rt-PCR) techniques to characterize molecular mechanisms underlying the protective effects of therapeutic hypothermia in CI-traumatized cochlear sensory epithelia from rats. Gene expression changes were confirmed at multiple time points between control, euthermic and hypothermia-treated implanted cochleae. Mild therapeutic hypothermia reduced inflammation and oxidative stress and increased activity of anti-apoptotic pathways, while preserving the blood labyrinth barrier. Moreover, flow cytometric analysis shows that hypothermia treatment significantly reduces the number of activated microglia, macrophages and leukocytes when compared with euthermic conditions. On-going research in our group is focused on identifying potentially synergistic therapeutics for combination with hypothermia and translating this technique to enhance protection of residual hearing and cochlear sensory epithelia.

Disclosures: **R. Sangaletti:** None. **E.A. Dugan:** None. **C. King:** None. **D. Dietrich:** None. **M. Hoffer:** None. **S. Rajguru:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.06. Auditory & Vestibular Systems

Support: EMBO ALTF 7-2017
FWO G0B2917N
BOF OT.14.118
NIH DC006212

Title: Sensitivity to Schroeder phase in octopus cells: Testing the dendritic delay hypothesis

Authors: *H.-W. LU[1], P. H. SMITH[2], P. X. JORIS[1];
[1]KU Leuven, Leuven, Belgium; [2]Dept. of Neurosci., Univ. of Wisconsin, Madison, WI

Abstract: Octopus cells in the mammalian cochlear nucleus generate remarkably well-timed responses to broadband transients. They reliably fire one spike per click (“entrainment”) for

trains up to 500 Hz, with temporal jitter <100μs. Their fast membrane time constants (<1ms) and apparent lack of inhibitory inputs make these cells ideal coincidence detectors, i.e. they only fire spikes when their auditory nerve fiber inputs are synchronously active. The traveling wave in the cochlea generated by broadband transients causes asynchronous activation of auditory nerve fibers such that fibers with high characteristic frequency (CF) are activated earlier than low-CF ones. This raises the question of how octopus cells reliably entrain to click trains. One hypothesis is that dendritic delay—EPSP travel time from the synapse to the soma—is longer for high-CF inputs than for low-CF inputs, thereby compensating for the cochlear delay and temporally aligning all inputs at the initial segment. This idea has so far only been tested in simulation studies.

Here we use Schroeder harmonic complexes to test this hypothesis in gerbil octopus cells in vivo. This harmonic complex can be regarded as repetitive brief frequency sweeps at the rate of its fundamental frequency. By varying the relative phase of each harmonic, one can generate a sequence of upward (positive Schroeder phase) or downward (negative Schroeder phase) frequency sweeps at different sweep rates. When all the harmonics have the same phase (zero Schroeder phase), the stimulus is similar to a broadband transient. According to the dendritic delay hypothesis, octopus cells should be maximally excited by zero Schroeder phase stimuli. The results, however, did not match this prediction.

Spike data from single unit recordings showed that each octopus cell not only can entrain at zero but also at other Schroeder phases. Moreover, there is a CF-dependent preference: 4 of 6 high-CF (>10 kHz) octopus cells entrain to positive phases, while 4 of 4 low-CF (< 5kHz) cells entrain to negative phases. Such preference is independent of fundamental frequency (from 50 to 400 Hz) or intensity (from 20 to 50 dB SPL) of the stimulus. Intracellular data shows that at zero Schroeder phase the cycle-averaged EPSP has a single large narrow peak. As the Schroeder phase deviates from zero, multiple EPSP peaks emerge in the cycle averaged response, to which the octopus can still show entrained spikes.

Taken together, we found that octopus cells not only entrain to broadband transients but also to periodic frequency sweeps. Our data suggests that there is a CF-dependent mechanism other than dendritic delays underlying such direction selectivity.

Disclosures: **H. Lu:** None. **P.H. Smith:** None. **P.X. Joris:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

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Program #/Poster #: 059.15/L22

Topic: D.06. Auditory & Vestibular Systems

Support: Schweppe Foundation and Armour Bequest/Rush Translational Sciences Consortium

The Children's Foundation

Title: Replication of the neural processing of pitch in youth without clinical diagnoses

Authors: *N. M. RUSSO-PONSARAN[1], A. KARLS[1], T. NICOL[2], N. KRAUS[2];
[1]Rush Univ. Med. Ctr., Chicago, IL; [2]Northwestern Univ., Evanston, IL

Abstract: Measurement of the frequency-following response (FFR) is a powerful tool for assessing the integrity of the auditory system and neural health. There is a growing literature suggesting the clinical utility of the auditory-evoked FFR and pitch tracking for conditions ranging from autism spectrum disorder to concussion. With the wide availability of portable neurophysiologic systems for capturing the auditory FFR, it is important to develop reliable, replicable protocols and normative data. In this pilot study, we aimed to replicate prior research (Russo et al., 2008) measuring the FFR to a speech sound /ya/ with a rising pitch contour (130-220 Hz, question intonation) in youth with no known clinical diagnoses (6-10 years old) and who had normal hearing based on click-evoked latencies and an auditory threshold screening. A primary goal was to show that, using a different system and within the confines of a small general office space, the FFR could be reliably collected in youth. A formal power analysis was not conducted. The FFR to the /ya/ was collected via three scalp electrodes using an Intelligent Hearing Systems' module. Two pitch-tracking variables analyzed in 2008, pitch error and pitch strength, were analyzed for the present data, as well as a new variable, correlation. Our data support collection of robust pitch-tracking responses in youth via our protocol. Both data from our full sample (M_{age} = 7.94 ± 1.4 years; n = 31, 17 boys) and a smaller sample more closely matched in age to the 2008 study (7-10 years; n = 21m 10 boys) yielded similar results (Table 1), with the exception of a large range of pitch error (.85 Hz – 32.57 Hz). Findings warrant further exploration to ascertain whether pitch error changes systematically with age, whether pitch error is a useful clinical measure, and whether a large error is an artifact of the collection system or a clinically significant result. While gender and age did not yield significant correlations or group differences in this sample, pitch error seems to decrease with age, while pitch strength and correlation seem to increase with age. Results warrant analysis in larger, well-characterized samples of youth across ages. With broader normative data, the clinical significance of neural pitch tracking will be clarified.

Table 1.

	6-10 years old n = 31 (2018)		7-10 years old n = 21 (2018)		7-13 years old n = 21 (2008)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Pitch Error (Hz)	11.08	10.05	9.96	9.08	8.52	2.20
Pitch Strength	0.53	0.18	0.55	0.18	0.39	0.20
Correlation (r)	0.67	0.34	0.68	0.33	n/a	n/a

Disclosures: N.M. Russo-Ponsaran: None. **A. Karls:** None. **T. Nicol:** None. **N. Kraus:** None.

Poster

059. Temporal and Spectral Auditory Processing

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Program #/Poster #: 059.16/L23

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 5T32LM012204-03

Title: Rhythmic auditory stimulation to entrain epileptic brain rhythms

Authors: *R. QUON[1], G. LESLIE[2], E. CAMP[3], B. C. JOBST[4];
[1]Geisel Sch. of Med. at Dartmouth Col., Hanover, NH; [2]Georgia Tech., Atlanta, GA;
[3]Dartmouth-Hitchcock, Lebanon, NH; [4]Neurol., Dartmouth-Hitchcock Med. Ctr., Lebanon, NH

Abstract: *Rationale:* Abnormal interictal epileptiform activity (IEA) is believed to contribute to many negative epilepsy-related health outcomes. The alarming percentage of drug-resistant epilepsy warrants the development of novel noninvasive therapies for modulating IEA. Our objective was to measure neural responses to a set of auditory stimuli containing enhanced 40-Hz spectral power, and to examine the effects that these auditory stimuli have on IEA.
Methods: Data was collected from intracranial electrocorticography (ECoG) patients at Dartmouth-Hitchcock Medical Center (DHMC). Subjects were adults with an average of 256 intracranial electrodes. Differences in the spectral perturbation and phase coherence were assessed between the stimuli groups. Interictal spikes were then identified with a template-based spike detector. The mean differences in baseline-normalized spikes were compared between the different stimuli groups, with the visual stimulus serving as the control. Nonparametric tests, such as the Kruskal-Wallis test for overall differences and the Mann Whitney U test for pairwise comparisons, were employed with corrections for multiple comparisons as appropriate (e.g. Bonferroni). Spike rate findings were then validated with linear mixed models.
Results: There was an increase in 40-Hz synchronization during active listening to the specifically engineered auditory stimuli versus baseline periods. The spectral contents revealed a significant difference in the 40-Hz band ($p < 0.05$) compared to baseline. Exposure to the pure 40-Hz auditory stimulus demonstrated a significant reduction in interictal spikes, in comparison to the visual control ($p < 0.05$).
Conclusions: This study demonstrates that select auditory stimuli can elicit gamma-band neural entrainment, and reduce IEA in persons with refractory epilepsy. Future studies should examine the therapeutic benefits that these specifically-engineered auditory stimuli have on epilepsy-related clinical impairments and should seek to define the optimal parameters for neural responses.

Disclosures: R. Quon: None. **G. Leslie:** None. **E. Camp:** None. **B.C. Jobst:** None.

Poster

059. Temporal and Spectral Auditory Processing

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Program #/Poster #: 059.17/L24

Topic: D.06. Auditory & Vestibular Systems

Support: FWO G0B2917N
BOF OT-14-118

Title: A temporal correlate to monaural edge pitch in the inter-spike interval statistics in the auditory nerve

Authors: *Y.-H. LI, P. X. JORIS;
KU Leuven, Leuven, Belgium

Abstract: Psychophysical studies show that the spectral edge of a bandlimited noise can create a pitch sensation, called “edge pitch”, which is close to but slightly mismatched (by a few %) to the actual edge frequency. For high-pass noise, the pitch is marginally higher than the edge frequency, while it is somewhat below the edge frequency for low-pass noise. The pitch sensation is usually speculated to reflect lateral inhibition (“Mach bands”) but could also reflect temporal factors caused by the frequency edge. We examine such factors in the auditory nerve.
We recorded spike trains from single auditory nerve fibers in the anesthetized chinchilla in response to high-pass and low-pass noise at a number of standard edge frequencies, as well as to broadband noise. For every stimulus, we compute the shuffled autocorrelograms (SACs) for all fibers, and average these to obtain the population interval distribution (PID). We then calculate the Pearson correlation coefficient between the PID and pulse patterns of different fundamental period to find the period best characterizing the interval most common in the auditory nerve. This period, the “estimated pitch”, is then compared with the subjective pitch reported in psychophysical studies.
The response of nerve fibers tuned near the edge frequency shows a high occurrence of interspike intervals close to the period of this frequency, which results in a local maximum in the correlation function between their SAC and pulse trains of different frequency. A periodicity close to the edge frequency also dominates the PID, resulting in a clear local maximum in its correlation with pulse trains of different frequency. As is the case perceptually, this local maximum (estimated pitch) tends to be displaced by a few percent away from the stimulus edge frequency towards the region of energy: towards a lower frequency for low-pass noise and higher frequency for high-pass noise. This displacement is larger for low-frequency than for high-frequency edges, again consistent with perception (Hartmann & McMillon, 2001).
We conclude that a temporal basis for edge-pitch is present in the auditory nerve in the form of a dominant period in the population inter-spike-interval distribution.

Disclosures: **Y. Li:** None. **P.X. Joris:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.18/L25

Topic: D.06. Auditory & Vestibular Systems

Support: DC016297

Title: General auditory and speech-specific contributions to cortical envelope tracking revealed using auditory chimeras

Authors: ***K. D. PRINSLOO**, E. C. LALOR;
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Abstract: Over the past few years research on natural speech processing has benefited from recognizing that low frequency cortical activity tracks the amplitude envelope of natural speech. This has been useful for investigating the mechanisms underlying speech processing, how such processing is affected by attention, and how audio and visual speech interact. However, it remains unclear to what extent these cortical measures reflect higher-level speech-specific processing versus lower-level processing of the spectrotemporal/acoustic stimulus dynamics. There has been somewhat equivocal evidence that speech intelligibility affects these envelope tracking measures, suggesting that they may indeed index speech-specific processing. These findings have led to the suggestion that different neural populations, having different functional roles in receptive speech processing, may simultaneously contribute to envelope tracking measures. In the present study, we aim to disentangle contributions to cortical envelope tracking that derive from general auditory processing of acoustic input from those that are functionally related to processing speech. To do so, we presented subjects with “auditory chimeras” - a previously introduced
technique that involves modulating the temporal fine structure (TFS) of one speech stimulus, with the amplitude envelope (ENV) of another speech stimulus. This can be done after splitting each stimulus into different numbers of complementary frequency bands, which allows a measure of control over which speech stimulus feature is actually recognized by the listener. We presented such chimaeric stimuli to subjects as we recorded their EEG. As we decreased the number of frequency bands in the chimaerae, subjects lost the ability to recognize the ENV speech stimulus and began to recognize some of the TFS stimulus. In line with this, the EEG tracking of the ENV stimulus dropped, but remained quite strong, and the EEG began to track the envelope of the TFS stimulus. These results highlight that, while cortical tracking of the speech envelope is largely driven by the acoustic energy of the sound, it contains contributions from speech-specific processing.

Disclosures: **K.D. Prinsloo:** None. **E.C. Lalor:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.19/L26

Topic: D.06. Auditory & Vestibular Systems

Support: NIH T32 NS047987

Title: Temporal perturbation releases auditory neural responses from adaptation

Authors: ***M. MENCELOGLU**[1], M. GRABOWECKY[2], S. SUZUKI[1];
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Abstract: The sensory systems utilizes temporal structures in the environment to build expectations about the timing of forthcoming events. Here, we investigated the effects of rhythmic temporal expectation on auditory perception. We tested how neural responses adapted to auditory rhythm and reacted to stimuli that violated that rhythm. In two experiments, we recorded scalp EEG while participants watched a nature video and passively listened to rhythmic tones with occasional temporal perturbations. In Experiment 1 (N=22) in the short-interval block, tones were frequently (80%) presented with 1s inter-tone-intervals (ITIs)—*short-standard* tones—and infrequently (20%) presented with 1.5s ITIs—*late* tones. Conversely, in the long-interval block, tones were frequently presented with 1.5s ITIs—*long-standard* tones—and infrequently presented with 1s ITIs—*early* tones. We analyzed the sensory evoked EEG responses including the auditory event-related potentials (ERPs) and theta/alpha inter-trial phase coherence (ITPC) recorded from midline frontocentral sites. The results revealed an early rate-dependent adaptation (*short-standard* < *long-standard*, greater response attenuation for the faster rhythm), as well as long-term adaptation based on the number of presented tones. Responses to the *early* tones yielded a near complete release from the early rate-dependent adaptation, whereas *late* and *long-standard* tones elicited comparable neural responses (*early ~ long-standard ~ late* > *short-standard*). In Experiment 2, we replicated our results using ITIs of 0.5s and 1s with a different group of 22 participants. These results suggest that (1) auditory sensory adaptation includes an early rate-dependent component and a long-term cumulative component, and (2) the early rate-dependent component may be subserved by mechanisms that generate temporal expectations because it was nearly eliminated by tones that violated rhythm-based expectations.

Disclosures: **M. Menceloglu:** None. **M. Grabowecky:** None. **S. Suzuki:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.20/L27

Topic: D.06. Auditory & Vestibular Systems

Title: Auditory steady state stimulation enhance gamma activity during sleep

Authors: *D. L. HENAO[1], G. HUBERFELD[2], J. F. NIETO[3], M. VALDERRAMA[4], M. LE VAN QUYEN[5];
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Abstract: Sleep oscillations are the expression of different physiological and cognitive processes taking place during sleep. These activities are associated with different essential functions in the brain as memory consolidation and synaptic reorganization. For this, nowadays there is a growing interest in modulating and enhancing these oscillations using non-invasive techniques. Previous studies have reported an increase in the expression of slow sleep oscillations and an improvement in memory retention after delivering in-phase auditory stimulation during sleep (Ngo et al., 2013). In addition, studies during wake states have shown an increase in cognitive behavior for pathologies as Alzheimer disease after a steady state gamma stimulation (Martorell et al., 2019). These changes, present in different neural networks, can be measured at the EEG level as evoked potentials that have different expressions according to the brain region and the excitability level. Despite this, the brain response to steady state auditory stimulation during sleep remains unclear. In this work, we studied brain responses to auditory steady state gamma stimulation (ASSGS) associated with different sleep stages. For this, we performed ASSGS experiments during complete nights in 6 adult subjects (mean age 23.5 years old) using white noise sound pulses of 1.5ms at 40 Hz during 400ms, triggered randomly across the night. Two nights (sham and stimulation conditions) were recorded by subject with the complete 10-20 international scalp EEG system and corresponding EOG and EMG polysomnographic signals. For the offline analyses, sleep scoring was performed and stimulation intervals were extracted from the recorded signals. Subsequently, gamma band power was compared between both conditions through a time-frequency analysis. Our results show an increase in the power of gamma band (40 Hz) during different sleep stages as a result of the stimulation events. The number of electrodes with a significant ($p < 0.05$) increase in gamma power was higher for N2 than for N3. The brain regions with increased responses included: frontal, central, temporal and occipital electrodes. These results point out to an increase of sleep gamma oscillations driven by auditory stimuli and raise the possibility of using this type of sounds in different pathologies and applications during sleep.

Disclosures: **D.L. Henao:** None. **G. Huberfeld:** None. **J.F. Nieto:** None. **M. Valderrama:** None. **M. Le Van Quyen:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.21/L28

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant AI129198

Title: Fluctuation in auditory test results in mice

Authors: ***T. M. MAKISHIMA**[1], B. YANG[2], R. COOK[2], J. MARUYAMA[2], S. PAESSLER[2]; [1]Univ. of Texas Med. Br. at Galveston, Galveston, TX; [2]Univ. of Texas Med. Br., Galveston, TX

Abstract: Mice have become an established model animal for studying the inner ear auditory and vestibular system due to its ease of genetic manipulations. Auditory testing in mice have been extensively done using methods such as auditory brainstem response (ABR), otoacoustic emission (OAE), or acoustic startle reflex (ASR). Although these methods have been studied in detail in association with different auditory function altering conditions, other minor factors that can affect the outcome are not always taken into consideration. For instance, most studies have traditionally not taken into account gender differences of the mice being tested, or less, on the gender of the human testers who manipulate the mice. Our goal was to determine whether there were gender differences in ABR or DPOAE in wild type C57BL6 mice. We tested wild type C57BL6J mice (n=10 each males and females) at age 6 weeks to 12 weeks. We performed ABR with 8, 16, 24 and 32 kHz tone pip and click stimulus and DPOAE with F2 value of 8kHz - 16kHz. We tested two sets of mice: first set (n=5 each males and females) were tested by a female tester first at six weeks of age, and then a male tester one week later. The second set (n=5 earch males and females) were tested by a male tester first at six weeks of age, and a female tester one week later. The results including ABR thresholds and amplitude of distortion products (DP) were compared using Student's t-test for statistical differences in set 1 vs set2, female mice vs male mice, and between testers. We also compared the quality of ABR waveforms. We observed significant ABR threshold difference between set 1 female mice and set 2 female mice with the male tester ($p<0.05$), set 2 threshold was smaller than set 1. Within both testers, there was no difference in ABR threshold between male and female mice. We observed significant DP amplitude differences between set 1 and set 2 female mice with the female tester ($p<0.05$). With the male tester, we observed DP amplitude difference between male and female mice in the low frequencies. With the female tester, we observed DP amplitude difference between male and female mice in the high frequencies. Overall, there was a trend of a larger DP amplitude difference between male and female mice with the female tester, of which the female mice had

larger DP amplitude. With the male tester, DP amplitude was similar between male and female mice. Taken together, there seems to be a slight difference in auditory test results depending on the gender of the mice, and also depending on the tester. However, we acknowledge that these results need to be interpreted cautiously.

Disclosures: T.M. Makishima: None. **B. Yang:** None. **R. Cook:** None. **J. Maruyama:** None. **S. Paessler:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.22/L29

Topic: D.06. Auditory & Vestibular Systems

Title: A new computational approach to the neural processing of sound, and it's about time

Authors: *S. K. SCOTT[1], K. JASMIN[2], C. F. LIMA[3];
[1]Univ. Col. London, London, United Kingdom; [2]UCL Inst. of Cognitive Neurosci., London, United Kingdom; [3]Univ. Inst. of Lisbon (ISCTE-IUL), Lisboa, Portugal

Abstract: There are functional and anatomical distinctions between the neural systems involved in the recognition of sounds in the environment and those involved in the sensorimotor guidance of sound production and the spatial processing of sound. Evidence for the separation of these processes has historically come from disparate literatures on the perception and production of speech, music and other sounds. More recent evidence indicates that there are computational distinctions between the rostral and caudal primate auditory cortex that may underlie functional differences in auditory processing. These functional differences may originate from differences in the response times and temporal profiles of neurons in the rostral and caudal auditory cortex, suggesting that computational accounts of primate auditory pathways should focus on the implications of these temporal response differences. In this poster we put forward this model in detail, and explore how how accounts for different empirical findings

Disclosures: S.K. Scott: None. **K. Jasmin:** None. **C.F. Lima:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.23/L30

Topic: D.06. Auditory & Vestibular Systems

Title: Region specific timescale dependency of auditory neural activity to song stimulus in avian brain

Authors: *M. INDA, A. YOSHIDA, K. HOTTA, K. OKA;
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Abstract: Zebra finches (*Taeniopygia guttata*) use their voices for communication. And song structures of individual males are important for sound recognition by females. The caudomedial mesopallium (CMM) and nidopallium (NCM) are known to be essential higher auditory regions for sound recognition and also these two regions have been discussed their fundamental functions and song selectivity. To clarify their functions and selectivity further, we investigated spiking patterns during song presentation. First, we applied Fano factor analysis to our spiking data. The Fano factor enables us to quantify spiking patterns for arbitrarily defined timescales. We calculated the changes of Fano factor as the function of time-window size (timescale) in the CMM and NCM to characterize the detail of firing patterns. While the changes in Fano factor for both regions were saturated at time-window sizes over 230 ms; at longer timescales it indicated clear different characteristics between two regions. Notably, the Fano factor for the NCM reached > 1, indicating that the spiking pattern of the auditory neurons in this region is a sub-Poisson process in short time windows but a super-Poisson process in long time windows. These results indicate that the spiking patterns of the auditory neurons in both regions differ depending on the timescale.Second, we developed a novel analysis named time-series correlation (TSC) to evaluate the contribution of neural activity to the temporal modulation of acoustic factors in the auditory neurons. The TSC can evaluate temporal neural activity and acoustic factors simultaneously. We calculated the TSC values for all seven acoustic factors as the function of time-window size, and observed a monotonic decrease in all seven acoustic factors. Because of constraints on the calculation of TSC, it is difficult to compare them accurately across different time windows. Therefore, to visualize the relative relationships between the changes in TSC values for all seven acoustic factors, we calculated the relative contribution of the TSC for each one to the total. This relative contribution changed with time-window size. Interestingly, the TSC for amplitude and wiener entropy were dramatically different from the control data, which were calculated from neural activity during silence.

Disclosures: **M. Inda:** None. **A. Yoshida:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.24/L31

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD 2R01DC012947-06A1
New York State ECRIP Fellowship
NIH NIMH P50MH109429

Title: Tracking rhythmicity of neural oscillations in the auditory thalamocortical system

Authors: *S. A. NEYMOTIN[1], A. BARCZAK[1], M. N. O'CONNELL[1], T. MCGINNIS[1], N. MARKOWITZ[2], E. ESPINAL[2], E. Y. GRIFFITH[3], S. DURA-BERNAL[3], W. W. LYTTON[3,4], S. R. JONES[5,6], S. BICKEL[2], P. LAKATOS[1,7];
[1]Ctr. for Biomed. Imaging and Neuromodulation, Nathan S. Kline Inst. For Psychiatric Res., Orangeburg, NY; [2]Neurosurg. / Neurol., Hofstra Northwell Sch. of Med., Manhasset, NY; [3]Physiol. & Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [4]Neurol., Kings County Hosp. Ctr., Brooklyn, NY; [5]Dept. Neurosci. & Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [6]CfNN, Providence VA Med. Ctr., Providence, RI; [7]Psychiatry, NYU Langone Med. Ctr., New York, NY

Abstract: A central debate about neural oscillations focuses on whether they occur continuously with amplitude fluctuations, or primarily as brief pulse-like events (e.g. event-related potentials). In the latter case, some argue that the presence of high spectral power is not sufficient to use the term oscillation, but that the definition depends on the underlying neural generators, and whether they are rhythmic or stochastic, in which case high power reflects specific temporal domain features. It is possible both hypotheses are correct, applying variously in different physiological frequency bands and depending on brain area or task. Quantification of specific signal features contributing to oscillations, such as number of cycles during high power activity, and measures of rhythmicity (coefficient of variation squared: CV2, Fano-Factor, lagged coherence) could help resolve these questions. To approach these questions, we quantified rhythmicity in two resting state (order of minutes) invasively recorded electrophysiology datasets: 1) simultaneous laminar electrode array local field potentials in nonhuman primate primary auditory cortex and medial geniculate body; 2) electrocorticography from human superior temporal gyrus. We extracted moderate/high power spectral events using Morlet Wavelets (4X median cutoff), determining event duration, peak frequency, number cycles (peak frequency x duration), and unfiltered waveform shape. All frequency bands had a wide/similar range of cycles/event, seen in unfiltered waveforms (1-24; median:3-4). We formed inter-event interval distributions and calculated CV2 (=1 is Poisson, < 1 is more rhythmic). CV2 increased with number of events in a time window, from longer windows of analysis, and for higher frequency oscillations, suggesting nonstationary inter-event interval distributions. To control for this, we varied window size for different frequencies (longer for slower frequencies) to produce similar number of events per window (N=16). All oscillations had a median CV2 (0.7 with end to start intervals, 0.5-0.6 with peak to peak intervals) and Fano-Factor (0.3-0.7 from delta to high gamma) consistent with rhythmicity. Lagged coherence, measuring phase continuity across epochs, was rhythmic across physiological oscillation frequencies (median 0.1-0.2). Narrow-band oscillations from 0.5-200Hz had higher lagged coherence (0.2-0.6, average 0.4).Our analyses demonstrate that both event-like pulses and rhythmic oscillations are widespread in thalamocortical dynamics. Further work is

needed to delineate circuit origins of the different processes and their behavioral/cognitive consequences.

Disclosures: **S.A. Neymotin:** None. **A. Barczak:** None. **M.N. O'Connell:** None. **T. McGinnis:** None. **N. Markowitz:** None. **E. Espinal:** None. **E.Y. Griffith:** None. **S. Dura-Bernal:** None. **W.W. Lytton:** None. **S.R. Jones:** None. **S. Bickel:** None. **P. Lakatos:** None.

Poster

059. Temporal and Spectral Auditory Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 059.25/L32

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD 2R01DC012947-06A1
NSF/Internet2 E-CAS grant

Title: Data-driven model of auditory thalamocortical system rhythms

Authors: *E. Y. GRIFFITH[1], S. DURA-BERNAL[2], A. BARCZAK[3], M. N. O'CONNELL[3], T. M. MCGINNIS[3], P. LAKATOS[3], W. W. LYTTON[2], S. A. NEYMOTIN[3];
[1]Neural and Behavioral Sci., [2]Physiol. and Pharmacol., State Univ. of New York Downstate Med. Ctr., Brooklyn, NY; [3]Ctr. for Biomed. Imaging and Neuromodulation, Nathan S. Kline Inst. For Psychiatric Res., Orangeburg, NY

Abstract: We used the NEURON simulator with NetPyNE to develop detailed biophysical computer models of the auditory thalamocortical system. We investigated the mechanisms and function of distinct types of neuronal oscillatory patterns observed in the auditory system in linear electrode array electrophysiological data recorded simultaneously from nonhuman primate primary auditory cortex (A1) and the medial geniculate body (MGB) of the thalamus, while the awake subjects were presented with different classes of auditory stimuli, including speech. Our initial model of A1 includes 6 cortical layers and multiple populations of neurons (3 excitatory types, 4 interneuron types). The A1 model projects to the thalamic model reciprocally to enable production of thalamocortical rhythms. The thalamic model includes the thalamic relay nucleus (MGB) and the thalamic reticular nucleus (TRN). MGB includes core and matrix populations with distinct projection patterns to different layers of A1. Model neurons have multiple ion channels which contribute to the different oscillations seen experimentally.
Cortical layer depth information was obtained from *in vivo* responses to a battery of sensory stimuli. L2-6 were populated with neurons, based on macaque cell density data. We used this information in conjunction with data on excitatory/inhibitory neuron ratios in layers 2-6 to estimate the number of excitatory and inhibitory cells/layer. L2-L6 interneurons were divided into 4 populations (somatostatin, parvalbumin, vasoactive intestinal peptide:VIP, and nonVIP)

with different synaptic connectivity patterns and time constants. Layer 1 inhibitory cells were included, since they have been identified as important targets of the thalamic matrix.
Our model demonstrated mechanistic origins of spatiotemporal neuronal oscillatory patterns observed *in vivo*. Some oscillations were intrinsic to cortex while others emerged from thalamocortical interactions: 1. inactivated core and matrix produced cortical theta (hence intrinsic to cortex); 2. activated core produced strong cortical alpha with reduced theta; 3. activated matrix led to moderate alpha with increased theta. The model demonstrated tradeoffs between different oscillatory states - particularly theta vs. alpha - consistent with experimental data. Further refinement of the model by including the results of detailed data analyses will enable predictions on how other brain regions contribute to different oscillatory dynamics. To confirm model predictions, we we will use targeted deep brain electrical microstimulation and pharmacological manipulations.

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Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.01/L33

Topic: D.06. Auditory & Vestibular Systems

Support: Knowles Leadership Fund
NIH grant 2T32MH067564.

Title: Characterizing the role of neuroligin 3 within cochlear ribbon synapses

Authors: *M. A. RAMIREZ[1], N. JONGKAMONWIWAT[2], J. N. SAVAS[3];
[1]Northwestern Univ. - Chicago, Chicago, IL; [2]Neurol., [3]Northwestern Univ., Chicago, IL

Abstract: Inner ear hair cells (IHC) are the primary mechanoreceptive cells within the cochlea and are innervated by auditory nerve fibers (ANF). The HCs house specialized structures called ribbons that allow for the faithful encoding of auditory information by mediating a graded release of glutamate upon HC depolarization. The released glutamate is then bound by AMPARs, causing an influx of cations into a single auditory afferent nerve fiber (ANF), ultimately triggering action potentials. The simultaneous depolarization of different ANFs then collectively relays information about the auditory stimuli into the brain. However, perturbations to these specialized cochlear ribbon synapses or impairments in their development result in decreased sensitivity to auditory stimuli. For instance, prolonged exposure to moderately intense auditory stimuli, like power tools, can result in the swelling of ANF terminals, retraction, and

eventual loss of ribbon synapses. Collectively, these perturbations are known as cochlear synaptopathy and are believed to result from the overstimulation of ANFs, which leads to excitotoxicity and the death or injury of ANFs. Cochlear synaptopathy thus refers to a type of noise-induced hearing loss, where the HC/ANF synapses are predominantly affected, which can result in permanent loss of auditory stimuli sensitivity.In a recent publication, we reported a draft of the cochlear proteome and identified a single synaptic adhesion protein, neuroligin-3 (Nlgn3), by mass spectrometry (MS) based proteomics. Trans-synaptic adhesion proteins are known to physically link pre- and postsynaptic membranes and regulate various signaling mechanisms during development. Specifically, Nlgns 1 and 3 are of interest as they localize to excitatory synapses, highlighting them as potential mediators of HC/ANF synapse development. Since our identification of Nlgn3 in the cochlea by MS, we have determined that ANFs express Nlgn3 by *in situ* hybridization and that the protein localizes to the ribbon synapses, by immunofluorescence. However, the significance of Nlgns being present at the cochlear ribbon synapses, and their potential role in hearing and noise-induced hearing loss, has yet to be investigated. Initial characterization of Nlgn3 KO mice has revealed significantly reduced levels of synaptic activity by auditory brainstem response recordings at moderate-high intensities of auditory stimulation. Overall, we aim to understand how Nlgn3 and potentially other Nlgns contribute to hearing by exploring their structural, physiological and molecular contributions to the ribbon synapse.

Disclosures: **M.A. Ramirez:** None. **N. Jongkamonwiwat:** None. **J.N. Savas:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.02/L34

Topic: D.06. Auditory & Vestibular Systems

Support: R01DC014712 to MAR

Title: Three dimensional electron microscopy of cochlear hair cell synapse morphology from hearing onset to maturation

Authors: S. PAYNE[1], N. SKIGEN[1], S. GATTANI[1], J. CARLQUIST[1], B. DAVIS[2], A. SCHWED[2], H. PATEL[1], *M. A. RUTHERFORD[1];
[1]Washington Univ. Sch. of Med. Dept. of Otolaryngology, St. Louis, MO; [2]Washington Univ. Sch. of Med. Program in Audiol. and Communication Sci., St. Louis, MO

Abstract: Cochlear inner hair cell (IHC) ribbon synapses excite auditory nerve fibers (ANFs) to transmit information about sound to the brain. Afferent synapses form during late embryonic and early postnatal stages. The synapses change morphologically during cochlear maturation through

~p30. Serial EM sections of the cat cochlea showed morphological differences among ribbon synapses and ANFs, suggesting a widely accepted classification scheme where ANFs on either side of the IHC have different morphologies, sound-response thresholds, and spontaneous rates (Liberman 1978, 1980). In mouse, the correlation between sound-response properties and synapse/ANF morphology is not established and serial EM has not been performed. Using a focused ion beam scanning electron microscope (FIB-SEM), we acquired ~$(30\ \mu m)^3$ regions of the IHC-ANF synapses from C57BL/6J mice at p17, when synaptic and spike generator structure and function are not entirely mature (Wong et al., 2014, Kim and Rutherford, 2016), and at p34, when the cochlea is functionally and anatomically mature. With 7nm isotropic voxels (X, Y, and Z) we segmented structures with AMIRA to reconstruct and measure ribbon synapses and ANFs to understand synaptic development and its role in maturation of hearing function. For spatial dependence of ribbon volume at p17, ribbons were larger on the modiolar-side ($4.9e^6 \pm 2.3e^6$ nm^3) than pillar-side ($4.4e^6 \pm 1.2e^6$ nm^3) when splitting the organ of Corti in two halves. In contrast, dividing each IHC into pillar and modiolar halves, regardless of IHC position in the organ of Corti, ribbon volume was larger on the pillar-side ($4.8e^6 \pm 1.1e^6$ nm^3) than modiolar-side ($4.5e^6 \pm 2.0e^6$ nm^3). We measured the distances between the outermost edge of the ribbon body to the synaptic cleft as an unbiased measure of synapse shape and size. The median distances between the ribbon and cleft of pillar-side synapses (335 ± 160 nm^3) were greater than modiolar-side synapses (256 ± 106 nm^3). Measuring the diameter of each afferent fiber, fibers synapsing onto the modiolar-side (1852 ± 162 nm^3) of the hair cell are smaller than those on the pillar-side (2087 ± 154 nm^3). Ribbons near the base of the IHC at p17 have the shortest nearest neighbor distances (2.67 ± 0.35 nm^3), smaller afferent fiber diameters, and smaller median distances between the ribbon and cleft compared to ribbon synapses elsewhere in the IHC. Comparisons will be made between p17 and p34.

Disclosures: **S. Payne:** None. **N. Skigen:** None. **S. Gattani:** None. **J. Carlquist:** None. **B. Davis:** None. **A. Schwed:** None. **H. Patel:** None. **M.A. Rutherford:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

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Program #/Poster #: 060.03/L35

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R00DC13107

Title: Alternative role of Sema3A/Nrp1 signaling during the morphogenesis of type I spiral ganglion neurons

Authors: ***H. L. CANTU**[1], M. R. PAPAZIAN[2], A. CACCAVANO[1], S. VICINI[3], T. M. COATE[2];

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Abstract: Proper connectivity between spiral ganglion neurons (SGNs) and hair cells in the cochlea is necessary for conveying sound information to the brain. The majority of SGNs are type I SGNs, which innervate inner hair cells (IHCs) and together these cells transmit the vast majority of sound input to the brain. Classic physiological and more recent single-cell RNAseq studies have shown that type I SGNs are divisible by morphology and synaptic location: thin type I SGNs with low rates of spontaneous discharge tend to innervate the modiolar side of the IHCs, and thicker type I SGNs with higher rates of spontaneous discharge tend to innervate the side of the IHC nearest the pillar cell. We have found that the supporting cells neighboring IHCs express *Sema3a*, while Nrp1 protein is detectable on SGN processes. Mutants that lack Sema3A signaling show a loss of the normal synaptic distribution of thin and thick type I SGNs at P2. Additionally, *Sema3a*mutants show disruptions in the normal distribution of presynaptic ribbon bodies and postsynaptic GluA receptors around the IHC at P30. Results from calcium imaging studies, where Sema3A gain- and loss-of-function experiments were performed, suggest that Sema3A may regulate SGN excitability. We are currently determining the extent to which Sema3A regulates type I SGN maturation and synaptic distribution by controlling SGN activity during development.

Disclosures: H.L. Cantu: None. **M.R. Papazian:** None. **A. Caccavano:** None. **S. Vicini:** None. **T.M. Coate:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.04/L36

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R15DC017866
Loyola University Chicago Faculty Start-Up Grant AU104818

Title: Spiral ganglion neurons with distinct preferred frequency responses employ different strategies to innervate the cochlear nucleus

Authors: S. S. MOHAMMED, A. J. PARNG, C. K. BORCEAN, H.-J. YOON, J. L. SCHEFFEL, D. A. GUTIERREZ, *W.-M. YU;
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Abstract: To allow animals to separate a complex sound into its frequency components, the auditory system is organized in tonotopy: neurons at various levels of the auditory pathway are

topographically arranged by their responses to different sound frequencies. Disruption of tonotopy often results in auditory processing disorders and language learning disabilities. Despite its importance in auditory functions and clinical implications, almost nothing is known about how the tonotopic map is established during development. In this study, we use genetic approaches to label spiral ganglion neurons (SGNs) and their auditory nerve fibers with different characteristic frequencies respectively. We found that functionally distinct SGN populations employ different cellular strategies to target and innervate neurons in the cochlear nucleus during tonotopic map formation. Auditory nerve fibers with high characteristic frequencies (high-CF fibers) initially overshoot and sample a large area of different targets before refining their connections to correct targets, while fibers with low characteristic frequencies (low-CF fibers) are more accurate in initial targeting and undergo minimal target sampling. Additionally, most high-CF fibers terminate on bushy cells unbranchedly as a single large Endbulb of Held, while some low-CF fibers form multiple terminal branching and small endbulb endings. These observations reveal the diversity of cellular mechanisms that functionally distinct auditory neurons use to pick their targets during tonotopic map formation.

Disclosures: S.S. Mohammed: None. **A.J. Parng:** None. **C.K. Borcean:** None. **H. Yoon:** None. **J.L. Scheffel:** None. **D.A. Gutierrez:** None. **W. Yu:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.05/L37

Topic: D.06. Auditory & Vestibular Systems

Support: MEXT KAKENHI 19H04743

Title: Morphological identification of zebra finch primary auditory neurons for parallel encoding of individually unique, but species-specific song features

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Abstract: Juvenile male zebra finches learn to sing by listening to conspecific adults. Male juveniles have to develop songs that are individually unique, while maintaining species-specific song characters. We recently found that juvenile zebra finches learn song syllables, but not silent gap patterns from other bird species. Further, we found two types of neurons that encode these song characters in parallel in the zebra finch auditory area called L3. Low-firing neurons (LF) selectively respond to syllable morphology and high-firing neurons (HF) respond to the temporal gap pattern of zebra finch songs. These neurons may form the substrate to develop unique, yet

species-specific songs (Araki et al. 2016). However, morphological characters of these neurons and how they are incorporated into the auditory neural network have not been well identified. Here we tried to identify LF and HF neurons morphologically by comparing them to morphological characters of L3 neurons which were elucidated by volumetric imaging in cleared tissue. By injecting adeno-associated virus vectors (AAVs) into the male zebra finch L3, we expressed different fluorescent proteins in L3 neurons under the pan-neuronal promotor, human synapsin (hSyn) and the inhibitory neuron-specific promotor mDlx. Fluorescent signals expressed with the hSyn or mDlx promotors showed little (5.3%) overlap. Furthermore, hSyn-positive neurons had significantly larger soma volumes (1.7×10^3 μm^3) compared to those of mDlx-positive neurons (1.3×10^3 μm^3), suggesting that the hSyn promotor was active predominantly in excitatory cells. hSyn-positive, putative, excitatory neurons were further classified into six morphological groups based on their soma volumes, dendritic arborizations, and axonal projection patterns. Two neuron groups with large soma sizes had enriched dendritic arborizations, and neurons in one of these groups project to multiple areas. Three neuron groups with intermediate soma sizes often had projections to a single area, while a neuron group with small somas rarely showed projections and had poor dendritic arborizations. We will further determine to which L3 neuron groups LF and HF neurons belong by registering morphological features of electrophysiologically identified LF and HF neurons.

Disclosures: M. Araki: None. **Y. Yazaki-Sugiyama:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: D.06. Auditory & Vestibular Systems

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MIDCD P30 DC005211
T32 DC000023
David M. Rubinstein Fund for Hearing research
John E. Bordley Professorship

Title: Sensitivity to tissue damage in mouse type II spiral ganglion neurons over the course of development

Authors: *N. J. NOWAK[1], M. B. WOOD[2], P. A. FUCHS[2];
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Abstract: Auditory information is conveyed to the central nervous system from the cochlea through type I and type II spiral ganglion neurons (SGNs). While the function of the type I SGNs is relatively well understood, definitive proof for the role of type II SGNs lags far behind. Limited recordings from type II SGNs demonstrate they are activated by near maximal outer hair cell stimulation, ATP, and by rupture of nearby outer hair cells. Taken together, these lines of evidence suggest that type II SGNs sense damaging levels of sound. However, because of intractability, recordings of type II SGNs have focused on the apical, low-frequency regions of excised Organs of Corti in pre-hearing animals. TH^{CreER}; GCaMP6f and $DrD2^{Cre}$; GCaMP6f mice were used to overcome some of these limitations by introducing a genetically encoded fluorescent calcium sensor in type II SGNs. Combined with two-photon microscopy techniques, these calcium sensors allow for observing real-time type II SGN activity that spans the Organ of Corti from apex to base in both pre-hearing and adult mice. In concordance with direct recordings, most type II SGNs are inactive without external stimuli but become more and more responsive with increasing levels of external potassium. Moreover, pre-hearing type II SGNs in both the apex and the base reliably experience calcium transients following hair cell ablation, recapitulating and extending previous electrophysiological recordings. Interestingly, however, early evidence suggests that in hearing adult mice, the same ablation procedures do not produce robust calcium responses. These findings could reflect less amplification of ATP signals from supporting cells following damage and/or reduced sensitivity of type II SGNs to ATP over the course of development. As such, the previously reported ATP sensitivity may be a transient developmental phenomenon and, if so, may challenge the notion that type II SGNs signal tissue damage by sensing ATP release from nearby damaged cells. Future study will explore activity of type II SGNs in adult mice before and after acoustic trauma.

Disclosures: **N.J. Nowak:** None. **M.B. Wood:** None. **P.A. Fuchs:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.07/L39

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC015901
NIH Grant GM103412

Title: Convergence of auditory nerve fibers onto globular bushy cells

Authors: *G. A. SPIROU[1], M. KERSTING[1], M. H. ELLISMAN[2], P. B. MANIS[3];
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Abstract: Globular bushy cells demonstrate enhanced synchrony to periodicity in the acoustic waveform relative to auditory nerve fibers (ANF), which provide their driving activation. Two very different mechanistic models to account for this property are the convergence of multiple subthreshold inputs that sum in a short coincidence detection time window, and convergence of multiple suprathreshold inputs, whereby the shortest latency input on each stimulus cycle generates a postsynaptic spike. To provide an accurate count and size measurement of these inputs, we imaged a portion of the GBC region of the cochlear nucleus at ultrastructural resolution, using serial blockface scanning electron microscopy (SBEM). This volume contained 27 complete GBC somata, which is the location of innervation by ANFs via modified endbulb terminals. Minimal stimulation in brain slices indicated 4-6 ANFs (5.1 ± 0.64 sd; Cao and Oertel, 2010) converged on single GBCs. Anatomical reconstruction of nerve terminals indicated a greater number (8.0 ± 2.03) of large terminals per GBC. Two patterns of innervation were noted: pattern #1 had 1-2 large and several moderately sized inputs (9 cells) and pattern #2 had only moderately sized inputs (7 cells), which suggest a mixed model of 1-2 suprathreshold and several subthreshold inputs, and a model of only subthreshold inputs, respectively. To assay the functional significance of these models, we generated swc files from GBC reconstructions, converted them into hoc code, and tested the effects of these two input patterns on a compartmental GBC model. Activation of individual inputs revealed that large inputs were suprathreshold and moderately sized inputs were subthreshold. We next generated spike patterns on afferent ANFs using a cochlear transduction model (Zilaney et al. 2014), and showed that model GBCs had enhanced first spike precision over their individual ANF inputs. Furthermore, model GBCs had enhanced vector strength in response to amplitude modulated CF tones over their ANF inputs (vector strength 0.66). Pattern #2 (mixed model) generated more synchronous (0.89) spike trains than pattern #1 (only subthreshold; 0.84), raising questions about the functional utility of mixed supra and subthreshold inputs.

Disclosures: **G.A. Spirou:** None. **M. Kersting:** None. **M.H. Ellisman:** None. **P.B. Manis:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.08/L40

Topic: D.06. Auditory & Vestibular Systems

Support: NIH F32 DC014878
NIH K99 DC016905
NIH R01 NS028901
NIH R01 DC004450
Hearing Health Foundation- Emerging Research Grant

Title: Synaptic actions of descending projections to the dorsal cochlear nucleus

Authors: *T. S. BALMER, L. O. TRUSSELL;
Oregon Hearing Res. Ctr. and Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: The dorsal cochlear nucleus (DCN) is a cerebellum-like circuit that integrates auditory nerve inputs with multisensory signals from non-auditory regions. In addition, the DCN integrates descending auditory signals from downstream areas of the auditory system. The inferior colliculus in particular—the main target of the DCN—sends a tonotopic projection back to the DCN. This descending projection has been proposed to have various roles in auditory processing, but without knowing the targeted cell types, and the postsynaptic responses in those cell types, the question remains how these signals are integrated into the DCN circuit and therefore what role they play in hearing. For example, descending projections to cells of the multisensory mossy fiber pathway (unipolar brush cells, granule cells and Golgi cells) could contribute to sound source localization or alternatively to suppression of self-generated sounds. Descending projections to principal projection neurons (fusiform or giant cells), or to neurons that inhibit them (cartwheel, stellate or vertical cells), could sharpen tuning through feedback excitation and lateral inhibition.
We labeled neurons in the DCN that receive descending projections from the inferior colliculus using an anterograde trans-synaptic viral approach in mice. We focused on the larger descending projection to the ipsilateral DCN. Unipolar brush cells of both ON and OFF subtypes, granule cells, and others, were trans-synaptically labeled from descending inferior colliculus neurons. We targeted these trans-synaptically labeled cells for whole-cell recording in acute brain slices that also expressed channelrhodpsin-2 (ChR2) in the descending axons. Specific activation of ChR2-expressing descending inputs showed that they were glutamatergic and were strong enough to drive action potential firing in UBCs and granule cells. The descending inferior colliculus–DCN projection, therefore, may modulate the firing of the parallel fiber pathway and could underlie mechanisms of multisensory processing. Other cell-types that were trans-synaptically labeled were also explored.

Disclosures: **T.S. Balmer:** None. **L.O. Trussell:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC004450 to LT
HHF Grant to TN

Title: Synaptic properties of a novel inhibitory cell type in the cochlear nucleus

Authors: *T. NGODUP, L. O. TRUSSELL;
Oregon Hearing Res. Ctr. and Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: In the ventral cochlear nucleus (VCN), feedforward inhibition from glycinergic cells is important for auditory processing by principal neurons in the VCN. However, only a single glycinergic cell type has been described in the VCN, the D-stellate or radiate cell. We used a well-characterized transgenic mouse line, GlyT2-EGFP, which labels all glycinergic neurons, to study the diversity of glycinergic cells in the VCN. With these transgenic mice, we discovered a surprisingly large population of GFP positive cells in the VCN that are distinctly different in somatic size, dendritic arbor and axonal projection from the well-known D-stellate cell class. We termed these cells “L-stellate” as their axons were short and terminated on local principal cells of the VCN. The cellular and synaptic properties of the L-stellate cell was studied using whole-cell patch-clamp recording of GFP-positive cells in brain slices. Stimulation of auditory nerve (AN) fibers generated both short (<1 ms) and somewhat longer (2-6 ms) latency EPSCs in the L-stellate cells, indicating that the L-stellate cells receive direct AN input and feedforward excitation from glutamatergic T-stellate cells in the VCN. However, when AN fibers were stimulated at high rates, the L-stellate cells showed prominent, delayed EPSCs occurring up to 100 ms after AN stimuli ceased. Because of the dual innervation of L-stellate cells, it is unclear of the delayed events are from the AN or the T-stellate cells, or both. In addition to the delayed EPSCs, we found that L-stellate cells showed prominent prolonged firing, lasting up to 100 ms following a period of high-frequency stimulation. This effect was synaptic in origin, as postsynaptic current injection produced trains of spikes that ceased immediately after the current step was over. Previous studies have suggested that the prolonged firing after high-frequency stimulation in some VCN neurons could be mediated by synaptic NMDARs, however, we were unable to block the delayed spiking with NMDAR antagonists, and are currently exploring other mechanisms. Nevertheless, the delayed firing of the L-stellate cells could explain the prolonged inhibition observed *in vivo,* and may be critical for sideband inhibition observed in primary excitatory neurons, bushy cells and T-stellate cells for the processing of auditory information.

Disclosures: T. Ngodup: None. **L.O. Trussell:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.10/L42

Topic: D.06. Auditory & Vestibular Systems

Title: Localization of cochlear nuclei by auditory nerve tracing in Trachemys scripta turtles

Authors: *J. R. DONOHUE[1], D. T. DALY[1], M. ARIEL[2];
[1]Dept. of Surgery - Ctr. for Anatom. Sci. and Educ. (CASE), St. Louis Univ., Saint Louis, MO;
[2]Pharmacol. & Physiol., St. Louis Univ. Sch. of Med., Saint Louis, MO

Abstract: The cochlear nuclei of turtles have been described in the brainstem medulla close to the root of the cranial eighth nerve (CN8) based on axonal degeneration techniques (Cruce and Nieuwenhuys. J. Comp. Neurol. 1974). As part of neurophysiological studies in red-ear pond sliders, we examined the localization of lagenar axons labeled with DiI in the lagenar branch of CN8 of intact fixed brainstems of three turtles. After 60 days for the DiI to diffuse within in the axonal membrane, transverse 30 µm cryosections were serially mounted onto subbed slides. . DiI profiles were observed rostrocaudally across 2 mm via confocal microscopy. The position and number of DiI profiles per mm2 were measured to evaluate the location of the ipsilateral cochlear nuclei. The proximity of the DiI profiles to DAPI-labeled neuronal nuclei were also examined. Surprisingly, there were more DiI profiles within one micron of a DAPI-labeled nucleus than further away, suggesting that many of the observed profiles could be making synaptic contact. Assuming that DiI remains in the axonal membrane and DAPI-labeled nuclei remain in the neuronal cytoplasm, close apposition of the DiI and DAPI label may be due to DAPI-labeled nuclei that are eccentrically located.. We find that the rostrocaudal distribution of the peak density of DiI profiles was near the caudal edge of the cerebellar peduncle, suggesting the possible center of the cochlear nuclei. There was a higher density of DiI profiles on the medial half of the brainstem, along the wall of the fourth ventricle, as compared to the lateral half of that wall. The profile density in the medial half was 98.3/mm2; the lateral half was 65.5/mm2. The subset of those data that have DiI profiles closely associated with the DAPI signal (possible synaptic contact) were also examined (medial half 75.7/mm2 and lateral half 52.3/mm2). Both data sets were found to be statistically significant with a p value < 0.05. . This study shows that the afferents of the lagenar branch of of CN8 of pond turtles are widely distributed within the fourth ventricle wall, with higher density medially at the level of the cerebellar peduncle. The appearance of DAPI-labeled nuclei that are not centered within their neuronal somas may be a unique feature of neurons of the cochlear nuclei.

Disclosures: J.R. Donohue: None. **D.T. Daly:** None. **M. Ariel:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.11/L43

Topic: D.06. Auditory & Vestibular Systems

Support: American Tinnitus Association
STINT/CAPES

CNPq

Title: CaMKIIa positive dorsal cochlear nucleus neurons are necessary for tinnitus perception in mice

Authors: *T. B. MALFATTI, B. C. BOERNER, R. N. LEÃO, K. E. LEÃO;
Brain institute, Federal Univ. of Rio Grande do Norte, Natal, Brazil

Abstract: Noise-induced tinnitus is a phantom sound, perceived without a physical source, caused by over-exposure to loud noise. The dorsal cochlear nucleus (DCN), a region known to integrate somatosensory and auditory pathways, has been identified as a potential key structure in the generation of phantom sound perception. Here, we decrease activity of the Calmoduline Kinase II alpha (CaMKIIa) positive DCN neurons to investigate their role in tinnitus perception. Mice were over-exposed to loud noise (90dBSPL, 1h, followed by 2h of silence) to induce tinnitus. Auditory brainstem responses (ABRs) and gap prepulse inhibition of acoustic startle (GPIAS) test were recorded two days before and two weeks after noise over-exposure to assure tinnitus induction (significant decrease in GPIAS response, $p = 0.003$, $n = 12$ mice) without permanent hearing loss. Activity of CaMKIIa+ neurons in the DCN was decreased by expressing and activating Gi-coupled human M4 Designer Receptors Exclusively Activated by Designer Drugs (hM4Di DREADD). Animals were retested on the following day in the GPIAS test but under effect of a systemic clozapine-n-oxide (CNO, 0.5mg/kg) administration. We found a decrease in tinnitus-like responses when CaMKIIa+ DCN neurons activity was decreased ($p = 0.018$, $n = 12$ mice), while the control group (control virus; CaMKIIa-YFP + CNO) showed no improvement in GPIAS responses ($p = 0.105$, $n = 6$). In another set of experiments, we administered CNO 30 minutes before the noise over-exposure to decrease activity of CaMKIIa+ DCN neurons ($n = 3$ experimental and 2 control mice) and preliminary results suggests that lowering CaMKIIa+ neurons activity can also prevent tinnitus induction. Our results suggests that CaMKIIa+ cells in the DCN may have a role in maintaining tinnitus perception in mice.

Disclosures: T.B. Malfatti: None. **B.C. Boerner:** None. **R.N. Leão:** None. **K.E. Leão:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.12/L44

Topic: D.06. Auditory & Vestibular Systems

Support: DFG SPP1608

Title: Effects of the two-pore potassium channel subunit TASK-5 on neuronal firing in the auditory brainstem

Authors: H. SABER[1], L. RÜTTIGER[2], M. KAISER[1], ***C. KÖRBER**[1];
[1]Univ. of Heidelberg, Heidelberg, Germany; [2]Univ. of Tübingen, Tübingen, Germany

Abstract: Auditory brainstem neurons are capable of ultrafast and precise information processing. This characteristic is a result of the ability of auditory brainstem neurons to fire action potentials at high frequency and fidelity. This ability develops over the first two postnatal weeks, before the onset of hearing, and is dependent on a precisely set neuronal excitability. Neuronal excitability in turn is determined by the resting membrane potential and the neuronal membrane resistance. The two-pore potassium channels (K2Ps) are voltage-insensitive potassium channels that form leak conductances which are crucial for both the establishment of the resting membrane potential and the membrane resistance. Of particular interest in terms of the setting of neuronal excitability in the auditory system is the K2P subunit TASK-5 which is selectively expressed in auditory brainstem neurons and upregulated around the onset of hearing. However, till date the functional role of TASK-5 is unknown. We examined the role of TASK-5 by knock-down and knock-out approaches in rats and mice. Acute reduction of TASK-5 expression by shRNA-mediated knockdown of TASK-5 mRNA in the ventral cochlear nucleus (VCN) and medial nucleus of the trapezoid body (MNTB) resulted in profound alterations in resting membrane potential and action potential waveform in both nuclei confirming the importance of TASK-5 for the regulation of high frequency, high fidelity firing. Conditional knockout of TASK-5 on the contrary did not result in a change in action potential waveform, but in a compensatory change in membrane resistance that limited effects of TASK-5 knockout to the processing deficits at high frequencies.

Disclosures: H. Saber: None. **L. Rüttiger:** None. **M. Kaiser:** None. **C. Körber:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

Support: Translational Research for Hearing Loss Grant from Action on Hearing Loss, London N1 1SE, United Kingdom
Pragma Therapeutics, Archamps, 74160, France

Title: Preclinical evaluation of a novel mGluR7 negative allosteric modulator in a noise-induced hearing loss mouse model

Authors: *R. M. AMANIPOUR[1,3], X. ZHU[2,3], B. DING[4,3], S. CELANIRE[5], G. A. DUVEY[5], A. SCHULTZ[4,3], R. D. FRISINA[1,2,3], J. P. WALTON[4,3];
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Hearing & Speech Res., Tampa, FL; [4]Dept. of Communication Sci. & Disorders, Univ. South Florida, Tampa, FL; [5]Pragma Therapeut., Archamps, France

Abstract: Hearing impairment is a common sensory disorder, affecting hundreds of millions of people worldwide. Over 30% of people over 60 have hearing loss. Noise induced hearing loss (NIHL) is characterized by cochlear dysfunction (inner ear), including increased hearing thresholds due to damage or loss of hair cells (HCs). Also, loud noise can cause swelling of auditory nerve fibers, linked to the excessive release of the neurotransmitter glutamate by hair cells; which is the main excitatory neurotransmitter in the auditory system. Effects of NIHL involves suppression of the amplitude, and elevation of thresholds of P1 peak (auditory nerve) and increases in the P2 peak (cochlear nucleus), of the auditory brainstem response (ABR). It then progresses, reducing cell density at the level of the spiral ganglion cells and cochlear nucleus (CN) of the auditory brainstem. Specifically, the dorsal (DCN) and anteroventral (AVCN) are thought to undergo plastic changes, possibly suggestive of altered inhibitory function as part of the progression of NIHL. Here, we investigated the effects of a novel negative allosteric modulator (NAM) on metabotropic glutamate receptor type 7 (mGluR7) on NIHL deficits in a mouse animal model: male CBA/CaJ mice with normal hearing at age 3-6 months. The mice were randomly placed in Control and Treated groups. The mGluR7 NAM was administered orally to the Treatment group at 1 hour pre-noise exposure and immediately following the exposure. All mice were exposed to an octave-band of noise at 110 dB SPL for 45 minutes. Five months post-exposure mice were sacrificed, and brain and cochleae were dissected and prepared for histology. Dramatic hearing preservation was seen for the Treatment group as measured with both distortion product otoacoustic emission (DPOAE) and ABR thresholds. Control mice showed signs of permanent threshold shift (PTS) 4 weeks post-exposure, which was prevented in the Treatment group. We also found evidence of improved neuron survival, including higher spiral ganglion neuron cell density in the Treatment group. Sound-exposed Control mice had significantly higher cochlear NFκB and AKT gene expression levels relative to the Treatment group, suggesting possible biological mechanisms for the development of PTS in the Controls. In order to evaluate the effects of inner HC/spiral ganglion excitotoxicity on CN function we are examining the possible relationships between functional synaptopathy as measured by ABR P1 amplitude and CN cell survival in the DCN and AVCN. These results will lay the groundwork for the first drug intervention involving mGluR7 blockade to prevent key aspects of NIHL.

Disclosures: R.M. Amanipour: None. **X. Zhu:** None. **B. Ding:** None. **S. Celanire:** A. Employment/Salary (full or part-time):; Pragma Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pragma Therapeutics. **G.A. Duvey:** A. Employment/Salary (full or part-time):; Pragma Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pragma Therapeutics. **A. Schultz:** None. **R.D. Frisina:** None. **J.P. Walton:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

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Program #/Poster #: 060.14/L46

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD RO1 DC004450
HHMI Gilliam Fellowship

Title: Distinct short-term plasticity of synaptic inputs onto auditory medial olivocochlear efferent neurons

Authors: *G. E. ROMERO, L. O. TRUSSELL;
Physiol. & Pharmacology/Oregon Hearing Res. Center/Vollum Inst., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The medial olivocochlear (MOC) reflex protects the auditory system from noise-induced hearing loss. While the MOC reflex has been studied extensively at the level of the cochlea, the central brainstem circuitry underlying its function is less well understood. This lack of knowledge hinders our understanding of the MOC system's protective role in regard to hearing loss, and its function during normal hearing, where it is dynamically active in response to a diverse auditory environment. This research aims to enhance our understanding of normal MOC reflex function by investigating the reflex' central circuitry. Here we report an analysis of ascending and descending excitatory synaptic inputs onto MOC neurons from the ventral cochlear nucleus (VCN) and inferior colliculus (IC), respectively. A ChAT-cre mouse line was crossed to a tdTomato reporter to generate mice (ChAT-tdTomato) with tdTomato in cre positive cells. In histological sections, labeled neurons were observed in the lateral superior olive and ventral nucleus of the trapezoid body (VNTB), where the somata of lateral and medial olivocochlear neurons reside, respectively. A retrograde tract-tracer was pressure injected into the membranous labyrinth of the inner ear and a majority of cre-positive neurons in the contralateral VNTB were labeled, confirming they were indeed MOC efferents. To enable optical excitation of ascending or descending terminals onto MOC neurons, the VCN or IC of ChAT-tdTomato mice were unilaterally infected with AAV2-CAG-ChR2-Venus-WPRE-SV40. To determine how MOC neurons integrate and convey synaptic information, we conducted patch clamp recordings on cre-positive VNTB neurons in acute brain slices from P30-P48 ChAT-tdTomato mice while optically exciting ascending or descending presynaptic inputs. Optogenetic activation of either input was found to evoke excitatory postsynaptic currents (EPSCs) in MOC neurons. These light-evoked EPSCs were due to inwardly-rectifying, fast-gating Ca^{2+}-permeable AMPA receptors. While postsynaptic AMPAR-mediated responses were similar between VCN and IC input, they differed in presynaptic short-term plasticity. Amplitudes of VCN originating

light-evoked EPSCs regularly depressed at low (5 Hz) to high (50 Hz) rates of stimulation, whereas IC originating EPSCs often facilitated. At high rates of stimulation, IC input exhibited presynaptic augmentation, as the recovery from facilitation was on the order of seconds. This suggests that ascending VCN input to MOC efferent neurons may best convey low rates or short bursts of action potentials, whereas descending IC input becomes stronger and more reliable at higher rates.

Disclosures: G.E. Romero: None. **L.O. Trussell:** None.

Poster

060. Auditory Processing: From Cochlea to Midbrain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 060.15/M1

Topic: D.06. Auditory & Vestibular Systems

Support: 1SC1MH086070-01

Title: Expression of glycine transporter 2 in the superior olivary complex

Authors: *J. FRAIRE[1], R. A. PEREZ[1], M. MIRANDA-ARANGO[2];
[1]Univ. of Texas At El Paso, El Paso, TX; [2]Dept. of Biol. Sci. and Border Biomed. Res. Ctr., Univ. of Texas at El Paso, El Paso, TX

Abstract: Glycine as a neurotransmitter is involved in the regulation of a variety of motor and sensory function such as vision, audition, breathing, and pain perception. Unlike GABAergic neurons which are well described in the brain, glycinergic pathways remain poorly explored. These glycinergic neurons are marked by the presence of glycine and the cell surface glycine transporters (GlyT1 and GlyT2). The goal of this project is to characterize the anatomical distribution of glycinergic projections from the Superior Olivary Complex (SOC), a GABA and glycinergic nuclei involved in audition. This aim will be addressed with the use of a transgenic mouse expressing the green fluorescence protein (GFP) under the promoter of GlyT2 (p-GlyT2-GFP) that allows identification of glycinergic neurons. Additionally, chemical and genetic tracers, in combination with immunostaining experiments, will allow a detail characterization of glycinergic projections and possible connections. Preliminary results suggest the presence of GlyT2 in green cells in the SOC and co-expression with GABA markers. Overall, this project will provide a better understanding of the distribution of glycinergic neurons in an auditory center and pave the way for functional and behavior experiments.

Disclosures: J. Fraire: None. **R.A. Perez:** None. **M. Miranda-Arango:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.01/M2

Topic: D.07. Vision

Support: NIH Grant R01-EY022411
University of Pennsylvania (University Research Foundation Pilot Award)
Hearst Foundations Graduate Student Fellowship

Title: The caudate nucleus controls coordinated patterns of adaptive, context-dependent adjustments to complex decisions

Authors: T. DOI[1], Y. FAN[2], J. I. GOLD[3], ***L. DING**[1];
[2]Neurosci. Grad. Group, Univ. of Pennsylvania, [1]Univ. of Pennsylvania, Philadelphia, PA; [3]Dept Neurosci, Univ. Pennsylvania, Philadelphia, PA

Abstract: Our decisions often need to balance what we observe and what we desire. However, our understanding of how and where in the brain such decisions are made remains limited. A prime candidate for integrating sensory observations and desired rewards, and a focus of many modeling studies, is the basal ganglia pathway, which is known to make separate contributions to perceptual decisions that require the interpretation of uncertain sensory evidence and value-based decisions that select among outcome options. In this study, we examined the roles of the caudate nucleus in mediating decisions that incorporate uncertain visual evidence and reward context information. As previously reported, we trained monkeys to perform a random-dot visual motion direction discrimination saccade task with asymmetric reward-choice associations. We obtained single-unit recordings from caudate neurons in two monkeys and found that a majority of these neurons (101/142) showed joint modulation by sensory evidence (motion coherence corresponding to at least one of the two choices) and reward (either the lateralized reward context or reward size) in at least one epoch. Of these neurons, 50 showed such combined modulation during motion viewing, with heterogeneous modulation patterns. To test for causal contributions of caudate neurons to decision formation, we perturbed caudate activity with electrical microstimulation in randomly interleaved trials (50% of trials) during motion viewing (n = 24 sessions for monkey C, 31 for monkey F). We found that caudate microstimulation evoked multiple effects on the monkeys' choice behavior and such effects often depended on reward context. Within a drift-diffusion framework, the microstimulation effects can be accounted for by changes in multiple parameters, suggesting that the caudate nucleus causally control multiple computational processes. Strikingly, the microstimulation effects on these parameters, particularly those that depended on reward context, reflected patterns of coordination in the monkeys' voluntary adjustments in response to asymmetric reward contexts. These results

imply that the caudate nucleus plays key roles in coordinating the deliberative decision process that balances external evidence and internal preferences to guide adaptive behavior.

Disclosures: T. Doi: None. **Y. Fan:** None. **J.I. Gold:** None. **L. Ding:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.02/M3

Topic: D.07. Vision

Support: NIH Grant R01-MH107620
NIH Grant R01-NS089521
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Uehara Foundation Research Fellowship
NARSAD Young Investigator Grant

Title: Neural mechanisms for flexible navigation-based decisions in mice

Authors: *S. KIRA[1], G. PICA[2], S. PANZERI[2], C. D. HARVEY[1];
[1]Harvard Med. Sch., Boston, MA; [2`]Istituto Italiano Di Tecnologia, Rovereto, Italy

Abstract: We studied the brain areas and activity patterns that are critical for transforming navigational signals into locomotor decisions. We focused on flexible behaviors, in which the same sensory information can lead to different actions depending on experiences stored in memory. To study flexible navigation-based decisions, we designed a delayed match-to-sample task for mice based on movement through a virtual reality T-maze. As mice ran through the T-maze, two cues were sequentially presented with a short delay (~1 sec) between them. The mouse was required to combine working memory of the first cue (sample cue) and visual information of the second cue (test cue) to make an appropriate action (right or left turn at the T-intersection). This task separates various types of processing that are often entangled in tasks with fixed sensory-motor associations. To identify cortical areas necessary for performance of the task, we bilaterally inhibited areas across the dorsal cortical surface by optogenetically activating ChR2-expressing interneurons. Inhibition of posterior parietal cortex, retrosplenial cortex, and visual cortices reduced behavioral choice accuracy. The effect was most prominent when these areas were inhibited during the trial phase in which mice had to combine visual and memory information to make an action. We recorded neural activity from these areas using two-photon calcium imaging during the task. Each area had heterogeneous activity patterns across the population, with individual cells having activity selective to visual cues, memory of cues, or

actions. In addition, a large fraction of cells had activity selective to a combination of the sample and test cues. The activity of these cells covaried with the mouse's choice accuracy, suggesting a potential role in accurate behavioral performance. These activity patterns could not be explained only by the measured running patterns of the mouse. Together, our experiments evaluated existing proposals for the functions of posterior cortical areas during navigation and decision tasks. We propose that a visual-parietal-retrosplenial network could be important for combining visual and memory signals to bias locomotor outputs and influence navigation decisions.

Disclosures: S. Kira: None. **C.D. Harvey:** None. **S. Panzeri:** None. **G. Pica:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.03/M4

Topic: D.07. Vision

Title: Frontal eye field neurons accumulate momentary vestibular acceleration and visual speed evidence for self-motion perception

Authors: *Q. K. ZHENG, L. ZHOU, Y. GU;
Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai City, China

Abstract: Accurate self-motion perception requires integration of momentary visual and vestibular signals with time-varying reliabilities. Previous research has mainly focused on the encoding process of these signals in sensory dominant areas including the dorsal portion of the medial superior temporal area (MSTd), the ventral intraparietal area (VIP), the parietoinsular vestibular cortex (PIVC), the posterior visual fissure (VPS) etc. However, little is known about how the sensory evidence from these areas is further decoded and accumulated in downstream areas like the saccade region of the frontal eye field (FEFsac) to optimize the decision process and guide self-motion. Here we recorded from 126 well-isolated single neurons in FEFsac in two macaque monkeys while they were performing a vestibular-visual heading direction discrimination task. We found that compared to the sensory neurons which typically present more homogeneous vestibular and visual tuning functions in the sensory areas, FEFsac neurons exhibit more mixed selectivity, including components from both sensory and task-relevant signals. Overall, FEFsac neurons show ramping activity in a way consistent with the drift diffusion model. In particular, FEFsac accumulates momentary vestibular evidence proportional to the acceleration profile, and visual evidence proportional to the velocity profile, suggesting that the decision related neurons in the frontal cortex selectively read out acceleration component from upstream vestibular signals. Although individual FEFsac neurons are heterogeneous, using the method of support vector machine (SVM) successfully discriminate heading directions from population of FEFsac activity, generating the performance pattern analogous to the monkeys'

behavior. In summary, our work suggests that similar to the posterior parietal lobe, the FEF may also accumulate vestibular and visual heading information to improve the precision of self-motion perception.

Disclosures: Q.K. Zheng: None. **L. Zhou:** None. **Y. Gu:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.04/M5

Topic: D.07. Vision

Support: JSPS KAKENHI Grant 17H01758
JSPS KAKENHI Grant 17K20020
JSPS KAKENHI Grant 23300102

Title: Changes in spontaneous brain activities during perception of 3-D object shape from motion

Authors: *S. IWAKI;
Natl. Inst. Adv Indust Sci. & Tech., Tsukuba, Japan

Abstract: Two-dimensional optical flow in retinal images is an important cue to perceive three-dimensional object embedded in the natural scene, which is called 3-D structure perception from motion (3DSFM). Brain mechanisms underlying 3DSFM had been studied by using fMRI and MEG (Orban et al., 1999; Iwaki et al., 2013), in which neural interaction between the dorsal and ventral visual systems appear to be critical (Iwaki et al., 2013). In this study, we manipulated parametrically the coherence of randomly moving groups of dots to create different levels of 3-D perception and to study the associated changes in brain activity, specifically, we focused on the correlation between neuroelectric activities and the confidence of 3-D object perception. Nineteen subjects participated in the study as volunteers. Visual stimuli consisted of 1000 randomly placed dots inside a spherical area (10 degrees in visual angle), which started to move 500 ms after the onset of presentation with various motion coherences. The coherence of the motion across dots varied from 0 to 100 %. A fully (100%) coherent stimulus had all the dots moving as if they belonged to a spherical surface rotating along a randomly tilted axis. On the other hand, the x % coherence stimuli contained (100 - x) % dots moving at the same speed as in the fully coherent stimulus but the movement directions of the dots were progressively randomized. During the interstimulus interval, the subjects were required to reply the tilt angle of the rotation axis and to rate subjective confidence of the answer by a 0 to 10 scale. The stimulus related EEG epochs of 2 s including 0.5 s pre-stimulus baseline, were recorded by using a 32-ch EEG system with a sampling rate of 1 kHz. The results of the correlation analysis between the

subjective rating of the confidence of the 3-D object perception and EEG time-frequency representation showed that there was significant positive correlation between fronto-parietal 40 Hz gamma-band power and the subjective confidence. In the previous studies, the relationship between the changes in transient gamma-band responses and the dopamine system in the human brain (Ahveninen et al., 2000) which biases the subjective confidence in the perceptual task (Andreou et al., 2015). The current results suggest that gamma-band synchrony in the fronto-parietal regions plays an important role in perceiving subjective confidence during 3DSFM.

Disclosures: S. Iwaki: None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.05/M6

Topic: D.07. Vision

Support: DARPA cooperative agreement HR0011-18-2-0019

Title: Characterization of the behavior of different neural network models performing a visual search task

Authors: D. A. NICHOLSON, *A. A. PRINZ;
Emory Univ., Atlanta, GA

Abstract: To investigate visual search and the cognitive computations that contribute to it, such as selective visual attention, scientists have long employed a now classic form of visual search task, in which subjects are shown a set of items and asked to report whether a target is present among distractors. Typically the task is used to identify factors that limit performance.
Recent advances in neural networks have led to a resurgence in their use as models of the brain. To better understand how neural networks compare to human subjects performing visual search, we studied the behavior of three models: (1) convolutional neural networks (CNNs), widely used in applied machine learning settings for their ability to classify images, and thought to function in ways analogous to the primate visual system, (2) a recurrent neural network model trained with reinforcement learning to make a series of glimpses (the Recurrent Attention Model (RAM) in Mnih et al. 2014, https://github.com/NickleDave/thrillington), and (3) large-scale spiking neural networks built with the Nengo simulator. We developed a small library to generate visual search stimuli (https://github.com/NickleDave/searchstims) for training and testing these models. We show that CNNs, unlike humans, achieve almost perfect accuracy on visual search, if training is performed correctly. Results obtained so far with the RAM model suggest it attains higher accuracy and behavior more similar to human visual search when its so-called glimpse sensor processes inputs in a way that is similar to how the eye processes the visual scene.

Experiments in progress compare the behavior of the RAM model with Nengo models that explicitly incorporate factors known to affect visual perception in humans, such as crowding effects.
Our results from training CNNs and RAM to perform visual search are consistent with recent studies suggesting that recurrent models better capture brain activity in the visual system than feed-forward models. We conclude that our findings support models of selective visual attention in the primate brain, which propose that visual processing is optimized not for classifying static images, but instead for integrating visual information across time.

Disclosures: D.A. Nicholson: None. **A.A. Prinz:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.06/M7

Topic: D.07. Vision

Support: NEI Intramural

Title: Stratal fast spiking GABAergic interneurons are necessary for object value learning based on the environment

Authors: *J. KUNIMATSU[1], O. HIKOSAKA[2];
[1]Univ. of Tsukuba, Tsukuba, Japan; [2]Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: In our daily life, the object values may change in different environments and we can choose different objects accordingly. We previously found that medium spiny neurons (MSNs) in the tail of the striatum (caudate and putamen) encode values of many objects stably. Interestingly, the MSNs change their value-coding flexibly depending on the scene environment (Kunimatsu and Hikosaka, SfN abstract 2017). How can the stable-to-flexible change occur? We then found that fast spiking GABAergic interneurons (FSIs) in the same regions are selectively sensitive to different scenes. This result raised the question: do FSIs cause scene-based value coding in MSNs?
To address this question, we examined the role of FSIs during the scene-based value learning task. In this task, monkeys viewed 2 groups of new fractal objects (A and B) in 2 familiar scenes (X and Y). In scene X, A-objects were associated with large reward, B-objects with small reward. In scene Y, B-objects were associated with large reward, A-objects with small reward. Since scenes X and Y were presented in a random sequence, the monkeys needed to switch the object choice abruptly depending on the scene. Yet, the monkeys became able to choose whichever objects were good after 160 trials. We recorded activity of MSNs and FSIs during the learning. We first found that MSNs developed object value-coding predominantly in 1 of the 2

scenes (e.g., A > B in scene X), while FSIs retained their selective responses to the scenes (e.g., Y > X). These results suggested that the scene-based object value-coding of MSNs is modulated by the inhibitory inputs from the scene-selective FSIs.
To test this hypothesis, we locally injected IEM-1460, an inhibitor of GluA2-lacking AMPARs, in the recording sites to selectively block the excitation of FSIs, but not MSNs. After the injection, monkeys were unable to learn new scene-object value association, suggesting that FSIs are necessary for the scene-based value learning. These MSN-FSI mechanisms may support the flexibly switching behavior based on stable value-coding in each environment: Flexibility based on Stability.

Disclosures: J. Kunimatsu: None. **O. Hikosaka:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.07/M8

Topic: D.07. Vision

Support: DST-INSPIRE Doctoral Fellowship

Title: Utilizing wisdom of crowds in the context of visual search task with varying difficulty levels

Authors: *T. SAHA ROY, S. MAZUMDER, K. DAS;
Indian Inst. of Sci. Educ. And Res. Kolkata, Mohanpur, India

Abstract: Collective decision making is often life-critical for different species of animals including humans. Group decision in humans has been shown to be beneficial for different complex cognitive tasks. One of the primary aspects of collective decision making is aggregating individual decisions into an optimal group decision. In real life humans often rely on majority rule to converge to a common decision. Our study consisted of a random target detection task in natural scenes, wherein 17 human subjects detected the presence or absence of a random target as indicated by the cue word displayed prior to stimulus display. The observers gave their response using a 10-point confidence rating. Concurrently their neural signals were recorded using a 64 channel EEG recording system. We demonstrate that weighted average of individual decision confidence produces significantly better performance than commonly used majority pooling of decision confidence levels ($p<0.001$). We also show that the group performance improves as a function of group size. Using multivariate pattern classifier, we analysed the neural signals as the observers performed the visual search task and also concurred that combining neural decision variables by simple averaging and weighted averaging results in a better combination strategy for random target detection using multiple brains. A separate

behavioural experiment was performed by 20 subjects on the same set of images to categorize the tasks according to their difficulty levels. The proportion of subjects rating a particular target detection task difficult determined its level of difficulty. We observe a gradual decrease in individual confidence of the subjects with increasing task difficulty. Further, we fit a statistical model on the individual confidence ratings and notice an increase in prediction error with increasing task difficulty ($r>0.9$, $p<0.001$). This error is significantly reduced ($p<0.05$ FDR corrected) upon combining the individual decisions using the group aggregation rules mentioned above. Using statistical tests, we show that combining all available subjects is unnecessary to achieve minimum prediction error. We conclude based on our results that averaging individual decisions often produces better performance and it is possible to predict an optimal group size for combination using complex real-life tasks varying in difficulty levels.

Disclosures: T. Saha Roy: None. **S. Mazumder:** None. **K. Das:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.08/M9

Topic: D.07. Vision

Support: German Research Foundation (DFG), DO 1240/3-1
DFG, SFB 936/A7

Title: Evidence accumulation in changing environments: Linking normative computation and neural implementation

Authors: *P. R. MURPHY[1], N. WILMING[1], D. HERNANDEZ BOCANEGRA[1], G. PRAT ORTEGA[2], T. H. DONNER[1];
[1]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Cortical Circuits Dynamics, IDIBAPS, Barcelona, Spain

Abstract: Perceptual decisions under uncertainty are commonly thought to result from the feed-forward, lossless temporal accumulation of the momentary ‘evidence’ for alternative world states provided by sensory cortex. An often-overlooked challenge of such decisions in natural environments is that the world state can itself undergo hidden changes, which requires adaptive tuning of the accumulation process to the environmental statistics. Here, we report that humans can approximate optimal decision-making in such changing environments, and that diagnostic signatures of the underlying computations are evident in the dynamics of motor cortex and produced by an established biophysical model of decision-making. Seventeen participants viewed sequences of visual evidence samples (dot locations) generated by one of two noisy sources, which could change during each trial with low probability. Their task was to report

which source was ‘active’ at the end of the trial. We combined MEG and source reconstruction to estimate decision-related neural population dynamics in regions of interest spanning visual, parietal, prefrontal, and motor cortex. Participants’ choices were consistent with the choices of the normative evidence accumulation process for the task. In particular, they gave especially strong weight to evidence samples that indicated with high probability that a change in the state of the environment had just occurred. Choice-specific preparatory activity in motor, premotor, and a movement-selective region in the junction of anterior intraparietal and postcentral sulcus exhibited the same sensitivity to change-point probability as the normative model and the participants, and encoded the model’s decision variable in a near-categorical fashion. These features were reproduced by a reduced version of a biophysical decision-making model with competitive attractor dynamics. In posterior intraparietal sulcus regions and early visual cortex, by contrast, we found hallmarks of evidence encoding and choice-specific feedback signaling. We propose that large-scale attractor dynamics in decision-related cortical activity approximate normative evidence accumulation in changing environments, and emerge from bi-directional information flow through sensory-motor pathways.

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Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.09/M10

Topic: D.07. Vision

Support: NIH R01EY019273-01

Title: Comparing the dynamics of neural response and choice probability in the frontal eye field and lateral intraparietal area during ongoing visual search

Authors: *K. MIRPOUR, J. W. BISLEY;
UCLA, Los Angeles, CA

Abstract: The lateral intraparietal area (LIP) and the frontal eye field (FEF) have been shown to play a significant role in attention and eye movement control. Neural correlates of many components of visual search have been investigated thoroughly in the context of simplified single step eye movements in these areas. Each area exhibits correlations to some sensory-motor elements of oculomotor decisions and many of these are similar between the two areas. Last year, we reported the division of labor in encoding and decoding the stimuli in the context of a naturalistic free eye movement behavior in these two areas. Here, we characterize the relationship between the dynamics of response modulations in the behavioral choice.

We recorded 431 (231 FEF and 200 LIP) neurons from 4 animals performing a free viewing visual foraging task. In this task, animals must find a target among five physically identical potential targets (T) and five task-irrelevant distractors. To receive the reward, the animal must fixate the target for 500 ms. The objects are arranged such that when the animal fixates one stimulus, another is likely to be in the response field of the neuron being recorded.
We quantified the response modulation of single neurons by the stimulus in the receptive field and at the fovea. This enabled us to calculate the dynamic modulation of the multiplexed responses to each stimulus and basic behaviorally relevant components at the level of individual neurons and the population. This analysis allows us to focus on the interactions between response modulations of different factors and their correlation to the behavioral choice. Although both foveal and receptive field stimuli were encoded with the different levels of strength and stability in the two areas, their pattern of co-modulating with the behavioral choices was fundamentally different. In LIP, only objects in the receptive field co-modulated with the choice, starting with fixation and staying stable to the end of the fixation. In FEF, the object in the receptive field co-modulated the response at the start of fixation, but toward the end of fixation, the foveal object, the receptive field objects and the object that would occupy the fovea in next fixation showed the same co-modulation with the choice. We conclude that LIP neurons contribute to the choice by representing a stable map of objects in the receptive field, whereas FEF neurons contribution to the choice involves a wide range of current and future objects in the fovea and receptive field that dynamically change in the time course of fixation.

Disclosures: K. Mirpour: None. **J.W. Bisley:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.10/M11

Topic: D.07. Vision

Support: NRF-2016R1A2B4011267

Title: Piano playing enhances awareness of musical scores during binocular rivalry

Authors: *S. GILL[1], S. KIM[2], C.-Y. KIM[1];
[1]Dept. of Psychology, Korea Univ., Seoul, Korea, Republic of; [2]Univ. of Sydney, Sydney, Australia

Abstract: When two dissimilar stimuli are presented dichoptically, perceptual dominance alternates between the two (Blake & Logothetis, 2002). During this phenomenon dubbed binocular rivalry, executing a simple and directly relevant action takes advantage in resolving the visual conflict (Beets et al., 2010; Maruya et al., 2007). In the present study, we investigated

whether a skilled motor action linked tightly to one of the two rival stimuli affects perceptual selection during rivalry when the linkage between action and perception is not concrete, but rather abstract. Seventeen skilled piano players (9.6 ± 0.93 years of training) dichoptically viewed a musical score scrolling from right to left and a vertical grating scrolling from left to right. There were three different response conditions for tracking the perceptual dominance of the musical score; In the "piano" condition, participants played the musical notes with a midi keyboard, the relevant motor action to the score. In the two control conditions, unrelated actions - pressing a computer keyboard button or discriminating the pointing direction of the stem of musical notes using two arrow keys (e.g., 'up' or 'down') - were assigned to the "binary" and "direction" conditions respectively. For all three conditions, the response tracking the vertical grating was identical, pressing a computer keyboard button. Due to the large individual variation of alternation rate during rivalry (Hancock et al., 2012), we normalized the dominance durations by dividing each score and grating dominance durations by the average of all dominance durations of each condition per participants. A repeated-measures ANOVA revealed a significant difference in normalized dominance duration of scores between the three conditions ($p<.001$). Post-hoc pairwise comparison showed that score dominance in the binary condition was significantly shorter compared to both direction ($p=.002$) and piano conditions ($p<.001$). More importantly in the comparison between direction and piano conditions, where both tasks required the tracking of each musical notes, score dominance of the piano condition was significantly longer than that of the direction condition ($p=.045$). These results suggest that during binocular rivalry skilled motor action extends the perceptual dominance of the relevant stimuli even when the action and perception are linked based on a high-level, symbolic association.

Disclosures: **S. Gill:** None. **S. Kim:** None. **C. Kim:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.11/M12

Topic: D.07. Vision

Support: NSFC Grant 31571117
NSFC Grant 31871101

Title: The loss function of perception is adjustable

Authors: ***T. TENG**[1,2], S. LI[3,4,5], H. ZHANG[2,3,4,5];
[1]Acad. for Advanced Interdisciplinary Studies, [2]Peking-Tsinghua Ctr. for Life Sci., [3]Sch. of Psychological and Cognitive Sci., [4]Beijing Key Lab. of Behavior and Mental Hlth., [5]PKU-IDG/McGovern Inst. for Brain Res., Peking Univ., Beijing, China

Abstract: Human perception is well modeled by Bayesian inference, where loss function is a necessary component that determines the optimal read-out from the posterior. Surprisingly, though often assumed to be quadratic, the form of perceptual loss function has rarely been empirically tested. Here we investigated a random-dot motion estimation task to infer the loss function of perception and whether it is modifiable. Each dot's moving direction was sampled from a skewed mixture of two Gaussian distributions such that we could manipulate the distance between the mean and mode of all dots' moving directions on a specific trial. L2 (quadratic) and L0 (hit = 0, otherwise = 1) loss functions predict perceptual read-out respectively at the mean and mode of the posterior distribution, which roughly corresponds to the vector-summation and winner-takes-all read-out in terms of neuronal population decoding. In Experiment 1 (N=15, 8 female, aged 18 to 25), we found the motion direction estimated by participants had a consistent deviation from the mean and biased towards the mode, with the bias increasing almost linearly (slope = 0.295 ± 0.044, $t(14) = 25.9$, $p < 0.001$) with the mode-to-mean distance, suggesting a loss function between L2 and L0. In Experiment 2 (N=2, both male, aged 22 and 24), we tested whether this default loss function can be modified by feedback that designates the mode as the correct answer. After three to four days' training, participants' estimation bias increased by 110% (164.1%, $t(286) = 12.4$, $p < 0.001$ and 55.1%, $t(286) = 3.9$, $p < 0.001$, respectively for the two participants) at the trained mode-to-mean distance level. Moreover, the post-training bias ratio (bias divided by mean-to-mode distance) at the untrained mode-to-mean distance level was similar to that of the trained distance level ($t(286) = -0.87$, $p = 0.38$ and $t(286) = 0.51$, $p = 0.61$, respectively for the two participants), ruling out possible visuo-motor remapping strategies. During both the training and test, we chose the mean and modal moving directions evenly across 360 degrees so that participants' estimation bias could not be explained by any prior belief either. We conclude that the loss function of perception can adapt to the external definition of loss. Given that feedback has been commonly used in perception studies as a means to specifically modify participants' prior belief, our finding that perceptual loss function can also be changed by feedback calls for a revisit of a variety of conclusions in the Bayesian framework.

Disclosures: **T. Teng:** None. **S. Li:** None. **H. Zhang:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.12/M13

Topic: D.07. Vision

Support: Brain R01: R01-NS104923
MURI: W911NF-16-1-0368

Title: Beta activity (15-30 Hz) modulates the choice probability in a visual selection task

Authors: *A. DUBEY[1], D. A. MARKOWITZ[1], B. PESARAN[2];
[2]Ctr. for Neural Sci., [1]New York Univ., New York, NY

Abstract: Our selection processes in day to day life are constantly shaped by the attention using two distinct processes: sensory-driven exogenous ("bottom-up") and goal-driven endogenous ("top-down") process. Exogenous attention is automatic and is captured by the saliency or novelty of the stimulus whereas endogenous attention is voluntary and can be engaged to select one thing over another. Besides these two, our selection process is also influenced by internal bias based on previous experiences. An important question is to understand how neural circuits encode the selection process. To address this, we recorded brain signals from the lateral prefrontal cortex (LPFC) of two adult monkeys (*Macaca mulatta*) while they performed a visual two-alternative choice task. Two iso-eccentric rectangular stimuli of different orientations (vertical or horizontal) were presented simultaneously. Luminance and relative reward values associated with the two targets were varied to manipulate the sensory and goal-driven attentional processes. Both monkeys showed a preferential bias for vertical target, further, the reaction times for selecting vertical target were significantly less from the horizontal target. Next, we studied the local field potential (LFP) and observed an increase in Beta band activity (15-30 Hz) during the baseline period before the Targets onset. Further, using the same framework as proposed by Markowitz et al. 2011 we found that power in Beta frequency range modulates the probability of target selection; specifically, low Beta power increases while high Beta power decreases the probability of selecting the anti-preferred horizontal target. Our results suggest the role of Beta activity in modulation of outcome of the visual selection process. **References:** Markowitz DA, Wong YT, Shewcraft RA, Pesaran B. Competition for visual selection in the oculomotor system. *J Neurosci.* 2011. 31(25):9298-306

Disclosures: A. Dubey: None. **D.A. Markowitz:** None. **B. Pesaran:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.13/M14

Topic: D.07. Vision

Support: NRF-2016S1A5A2A01023762

Title: Brain activity associated with preferences for artworks depending on the context of human or AI creators

Authors: *S. NAM, J. SONG, C.-Y. KIM;
Dept. of Psychology, Korea Univ., Seoul, Korea, Republic of

Abstract: Previously, it has been shown that aesthetic judgments of an artwork depend on contexts, such as the authenticity (Newman et al., 2011), or the display place (Kirk et al., 2009). In the present study, we aimed to examine whether the contextual information of a creator - i.e., the human or the artificial intelligence (AI) - influences the viewers' preference judgments of an artwork. In addition, we monitored the viewers' brain activity by using functional magnetic resonance imaging (fMRI). 54 images of Impressionist landscape paintings were selected as human-made artworks. The other 54 images were created by Google 'Deep Dream Generator', mimicking the Impressionist style through deep learning algorithms. 36 participants performed a preference rating task inside the MRI scanner on each of the 108 artworks accompanied by one of the two creator labels. There was no statistically significant main effect of the creator labels (ANOVA, $F(3,32) = 1.02$; $p = 0.31$) across all the participants. However, the participants were divided into the two sub-groups -i.e., human-preferring or AI-preferring groups - based on their initial relative preference for the creator information. The human-preferring group showed the main effect of the creator labels in the posterior cingulate cortex bilaterally which is a part of the Default Mode Network implicated in the internal process while appreciating the artwork (Vessel et al., 2012). The main effect was also found in the left lingual gyrus and the left anterior culmen both of which are involved in visual aesthetic experience (Boccia et al., 2015). In contrast, the AI-preferring group showed no clusters that showed the statistically significant main effect of the creator labels. These results suggest that the contextual information of the creator has an effect on the aesthetic preference judgments of the viewers, which was reflected in the differential activation patterns in the brains of the viewers based on their initial creator preference.

Disclosures: S. Nam: None. **J. Song:** None. **C. Kim:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.14/M15

Topic: D.07. Vision

Support: NIH Grant DA018926
NSF-GRFP
NIH Grant EY021462

Title: Incentivizing dissecting and modeling human confidence judgments

Authors: *Z. BOUNDY-SINGER, C. M. ZIEMBA, R. L. T. GORIS;
Ctr. for Perceptual Systems, Univ. of Texas At Austin, Austin, TX

Abstract: Perceptual systems interpret the environment on the basis of sensory information that is routinely impoverished and noisy. Observers are aware of the fallibility of perception and can

introspect about the correctness of a perceptual decision. What computations underlie confidence in a decision? One possibility is that confidence simply reflects the magnitude of sensory experience. Alternatively, confidence results from a more principled computation that takes uncertainty about the stimulus into account. To test these hypotheses, we developed a novel decision-making paradigm. In our task, observers jointly report a categorical judgment of a sensory stimulus and their confidence in this decision. Critically, rather than relying on observers' subjective rating of confidence, we provide them with an objective incentive. Correct high confidence decisions result in a big monetary reward, while incorrect high confidence decisions result in a big cost. To maximize total reward, reported confidence must reflect the probability of being correct. All alternative strategies are less profitable.
We conducted two novel experiments. In the first experiment, we tasked observers to judge the orientation of noisy visual stimuli and varied both stimulus strength and stimulus uncertainty. We found that reported confidence was strongly indicative of task performance. Moreover, confidence judgements depended both on stimulus strength and stimulus uncertainty, ruling out the hypothesis that confidence simply reflects the magnitude of sensory experience. In the second experiment, we tasked observers to judge the orientation of noisy visual stimuli and varied the reward scheme to incentivize either a risky or a safe confidence-reporting strategy. We found that the incentive strongly affected the proportion of high-confidence judgments, but did not change perceptual performance.
Can a single model account for both sets of results? We fit each observer's data with a two-stage decision-making model in which the perceptual report results from comparing the magnitude of a noisy sensory response with a decision-criterion. The confidence report results from comparing the magnitude of the normalized sensory response with a confidence-criterion, whereby the normalization term is a noisy estimate of the sensory noise. We found that this model captured our data well. Together, our findings suggest that perceptual confidence can be most fruitfully studied as arising from a principled computation that reveals information about the quality of the perceptual decision, but is itself subject to a decision-making process.

Disclosures: **Z. Boundy-Singer:** None. **C.M. Ziemba:** None. **R.L.T. Goris:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.15/M16

Topic: D.07. Vision

Support: Internal funding

Title: Distinct BOLD signal time course profiles found for sensation, reward, and perception in social alcohol drinkers

Authors: *K. SANDERS, T. W. JAMES;
Indiana Univ., Bloomington, IN

Abstract: Considerable evidence suggests sensory systems are entrained by substance-induced rewards, resulting in perceptual sensitization to specific substance-related sensory cues (Hanlon et al., 2015; 2010; Franken, 2003) and creating an unavoidable pathway to automatic substance seeking behaviors. Drug-related sensory cues produce heightened activation in addicted individuals in brain regions that integrate early sensory information, and the degree of this atypicality positively correlates with addiction severity and can predict craving and relapse (Yalechkov et al., 2010; Marhe et al, 2013). An ideal candidate task for the investigation of atypical sensory processing is perceptual decision-making. Perceptual decisions require accumulation and integration of sensory evidence, which is influenced by a stimulus' perceptual history, which in turn is dramatically impacted by the reward value associated with that stimulus. A large body of evidence suggests that each of these constructs influence activity in different brain regions and affect different aspects of evidence accumulation (Ploran et al, 2007; Carlson et al., 2006; Hampton & O'Doherty, 2007).
Using functional magnetic resonance imaging, we designed an event-related decision task that slowed visual processing through use of a gradual reveal. Participants passively viewed an image of alcohol or electronic devices as the background receded in a checkerboard and were instructed to indicate with a button press when they were able to identify the image. After a delay, participants then made a behavioral decision about how likely they were to engage with the image based on a hypothetical situation. Images presented were individuated to participants' pre-existing levels of experience with and exposure to the items. BOLD signal time courses were extracted from a-priori ROIs and interpolated to align with average recognition time.
We have found that sensory evidence, perceptual history, and reward information all leave distinct footprints in the BOLD signal time courses of evidence accumulation, onset, and rate in function-specific neural regions. Our work details the temporal ordering of the influence of sensory evidence, perceptual history, and reward value. To our knowledge, the neural time course of reward processing has not been investigated and our methodology has uniquely localized and characterized perceptual accumulation across many different brain regions. These findings have direct applicability to cue-reactivity treatment approaches, which would directly benefit from more explicit sensory processing management protocols.

Disclosures: K. Sanders: A. Employment/Salary (full or part-time):; Indiana University. **T.W. James:** A. Employment/Salary (full or part-time):; Indiana University.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.16/M17

Topic: D.07. Vision

Support: HHMI
NIH Grant EY11378
ADRC Pilot Award
Simons Foundation SCGB 414196
NARSAD Young Investigator Award
NIH Grant EY018849

Title: Deficits in decision making after pharmacological and chemogenetic inactivation of Area LIP

Authors: D. JEURISSEN[1], *S. SHUSHRUTH[1], Y. EL-SHAMAYLEH[1], G. D. HORWITZ[2], M. N. SHADLEN[3];
[1]Columbia Univ., New York, NY; [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [3]Neurosci., Howard Hughes Med. Inst. - Columbia Univ., New York, NY

Abstract: Many neurons in the lateral intraparietal area (LIP) exhibit spatially selective persistent activity for planned saccades. Such persistent activity has been shown to be a substrate for computations like accumulation of evidence for perceptual decisions reported by saccades. Electrical stimulation of LIP causes more and faster decisions in favor of contralateral choice targets, indicating a causal role for LIP in decision making. However, pharmacological inactivation of LIP has been reported to exert no effect on perceptual decisions. We hypothesized that a null effect could arise from incomplete inactivation of LIP or compensation by other brain areas. LIP contains redundant representations of contraversive saccades, and other brain areas (e.g., FEF, area 46 and Superior Colliculus) are known to represent evidence accumulation for perceptual decisions. To test our hypothesis, we characterized the extent of persistently active neurons in the lateral bank of the intraparietal sulcus in two monkeys and used this to guide inactivation. We inactivated LIP either with multiple injections of the GABA agonist muscimol or by chemogenetic inhibition.
Monkey 1 was trained to discriminate the direction of random dot motion, viewed in the right hemifield, and to report its decision with an eye movement to a left or right choice target. Inactivation of right LIP by muscimol injection affected decisions mainly by biasing choices to the ipsilateral target. Within a session, the bias dissipated after ~200 trials (~20 min). Further, the size of the ipsilateral bias decreased over consecutive inactivation sessions. Injection of the vehicle (interleaved sessions) did not lead to an ipsilateral bias.
Monkey 2 was trained to discriminate the order of appearance of two targets asynchronously flashed in opposite hemifields. After an imposed memory delay, the monkey reported the remembered location of the first target with an eye movement. We expressed the inhibitory receptor hM4D(Gi) in the right LIP. Systemic administration of the receptor activating drug clozapine resulted in a dose-dependent bias for the ipsilateral target. The bias was again strongest in the first ~200 trials and weakened after that.
Our findings reaffirm that neural activity in LIP likely has a causal role in perceptual decision making. However, compensatory mechanisms can be recruited within minutes of its inactivation, resulting in recovery of behavior. The mechanism of compensation for neural inactivation is not

known. However, the finding invites caution in the interpretation of null inactivation effects, especially in complex, redundant association areas, which do not pose a singular bottleneck.

Disclosures: **D. Jeurissen:** None. **S. Shushruth:** None. **M.N. Shadlen:** None. **Y. El-Shamayleh:** None. **G.D. Horwitz:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.17/M18

Topic: D.07. Vision

Support: NIH BRAIN Initiative grant R01 EB026949

Title: Unbiased estimation of firing-rate variance from spikes to reveal decision computations

Authors: *C. AGHAMOHAMMADI, T. A. ENGEL;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Behavioral responses during many forms of decision-making (e.g., accuracy and reaction times) are highly consistent with the accumulation of noisy evidence towards a decision bound. How this evidence accumulation is implemented in single neurons is an open question under active debate. Neurophysiological studies of decision-making demonstrate that trial-averaged firing rates in several brain regions ramp up or down, which has been widely interpreted as a neural correlate of evidence accumulation on single trials. However, ramping in trial-averaged firing rates can emerge from many different kinds of single-trial firing rate patterns, consistent with alternative models of decision computation. To differentiate among these alternatives, several recent studies used a method of partitioning total variance of spike-counts of single neurons into two fundamental components: the variance of the spike-generation process and the trial-to-trial variance of the underlying firing rate [1]. The firing-rate variance inferred with this method ramps linearly during decision formation, supporting noisy evidence accumulation by single neurons on single trials.
Here, we re-examined the assumptions of the previously used method of partitioning spike-count variance and discovered statistical flaws, the consequences of which can be detrimental to the interpretation of inferred neural computations. Through theoretical analysis and simulations, we found that the previous method has two sources of bias affecting the estimation of firing-rate variance. The first source arises due to the finite number of trials available for estimation and leads to systematic underestimation of the rate variance. The second source arises due to the finite size of time-bins and leads to systematic overestimation of the rate variance. Either of two sources can dominate for different ground-truth rate variance, trial number and bin size, leading to unpredictable estimation biases. Moreover, for a biologically realistic trial number, the bias

can produce an artifactual rise of the estimated rate variance, when the average firing-rate increases and the ground-truth variance is constant. To overcome these problems, we developed a new estimation method, which explicitly accounts for the dependence of spike-count variance on the bin size. By comparing spike-count variance in time-bins of different size, our method produces an unbiased estimation of the firing-rate variance with the finite number of trials. In the future, our new method can be used to re-evaluate decision-related activity of single neurons on single trials.
[1] Churchland et al., Neuron, 2011.

Disclosures: C. Aghamohammadi: None. **T.A. Engel:** None.

Poster

061. Decision Making I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 061.18/M19

Topic: D.07. Vision

Title: Value computation in a naturalistic foraging task by non-human primates

Authors: *B. CAZIOT[1], P. R. SCHRATER[2], X. S. PITKOW[3], D. E. ANGELAKI[1];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Univ. Minnesota, Minneapolis, MN;
[3]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Foraging is a wide-spread natural behavior that requires integrating expected rewards with action costs. In the lab, foraging is usually studied using proxy-tasks with unclear ecological validity. Here we studied how macaques perform this computation in a naturalistic setting by training animals (N=3) to navigate within a virtual environment to forage for rewarding targets. Two targets briefly appeared at the same time, each providing a color cue that indicated variable rewards from 0 to 0.3mL of juice. The targets were uniformly distributed over a wedge of locations in the environment, within a range of angles [-22.52, +22.5deg] and distances [50, 400cm] from the observer. The virtual ground plane was shown by a dynamic pattern of small random triangles with varying density (1 or 50 dots/m-2). Monkeys had to decide which of these two targets to acquire, and then navigate through the environment using a joystick to stop within a radius (75cm) of the memorized target location. The monkeys preferred higher value targets, as well as closer and more straight-ahead targets. Critically, they made decisions based on a combination of target position and reward value, and were less sensitive to the effect of reward value when floor density was low. To maximize expected value, we hypothesized that monkeys were sensitive to travel time, control cost and accuracy. We estimated these quantities for each target position based on the behavior, and assumed monkeys represent these quantities. We then performed multivariate logistic regression of the monkeys' decisions from the difference between various parameters for the 2 targets. We found that all variables (distance, angle, travel

time, control cost, accuracy and reward) except floor density contributed to the monkeys' decisions. We found no interaction between variables besides floor density. Overall our results show that monkeys integrate multiple sources of information, including estimates of action costs, to compute the value of alternative actions.

Disclosures: B. Caziot: None. **P.R. Schrater:** None. **X.S. Pitkow:** None. **D.E. Angelaki:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.01/M20

Topic: E.01. Eye Movements

Support: NSERC

Title: A post exercise benefit to executive function is independent of a change in cerebral blood flow: Evidence from hypercapnia

Authors: *B. TARI, J. VANHIE, M. A. FADEL, G. R. BELFRY, M. HEATH;
Sch. of Kinesiology, Fac. of Hlth. Sci., Univ. of Western Ontario, London, ON, Canada

Abstract: A single-bout of moderate aerobic exercise for as little as 10-min improves executive function. Notably, however, the neurophysiological basis for this post-exercise improvement remains unclear. One proposed mechanism is an exercise-induced increase in regional cerebral blood flow (CBF) to an extensive frontoparietal network. To determine if CBF - in part - supports the post-exercise benefit to executive function, participants completed four separate experimental conditions. In the first condition, participants completed a VO_{2peak} test to determine cardiorespiratory fitness. The second condition entailed a 10-min exposure to a hypercapnic environment - achieved by inhaling a gas mixture containing a higher-than-atmospheric concentration (i.e., 5%) of CO_2. The hypercapnic condition was employed because it results in an increase in cerebral blood flow similar to an exercise manipulation. The third condition entailed 10-min of aerobic exercise (via cycle ergometer) at an intensity that produced a change in CBF velocity equivalent to the hypercapnic condition. The fourth condition involved a 10-min non-exercise condition in which 'normal' air was breathed (i.e., control condition). During the hypercapnia, exercise and control conditions, blood flow velocity and hemoglobin deoxygenation were measured via transcranial doppler ultrasound (TCD) and near-infrared spectroscopy (NIRS), respectively. Pre- and post-condition executive function was determined via the antisaccade task. Antisaccades are non-standard goal-directed eye-movements requiring a saccade mirror-symmetrical to a target stimulus, and are mediated via frontoparietal networks that show task-related modification following single-bout and chronic aerobic exercise. Results

showed that post-exercise antisaccade RTs reliably decreased, whereas hypercapnia and control condition RTs did not. Accordingly, the metabolic changes associated with exercise - and not a change in cerebral blood flow - result in a post-exercise executive benefit.

Disclosures: B. Tari: None. **J. Vanhie:** None. **M.A. Fadel:** None. **G.R. Belfry:** None. **M. Heath:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.02/M21

Topic: E.01. Eye Movements

Support: Beckman Institute for Advanced Science and Technology
Center on Health Aging and Disability

Title: Voluntary saccade training of large amplitudes in healthy older adults yields bilateral changes in latency and kinematics

Authors: *P. B. CAMACHO[1], R. CARBONARI[2], C. LOPEZ-ORTIZ[3];
[1]Neurosci. Program, Univ. of Illinois at Urbana Champaign, Urbana, IL; [2]Beckman Inst. for Advanced Sci. and Technol., [3]Kinesiology and Community Health, Neurosci. Program, Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Voluntary saccade function is key for visual navigation of the environment. Decreased function of voluntary saccades correlate to freezing of gait in Parkinson's disease. We tested the effects of training voluntary saccades in eight cardinal and intercardinal directions in five older adults (ages 40-65) with no known neurological disorders. Voluntary saccades were performed to targets at 10°, 20°, 30°, 40°, 45°, and 50° from a central fixation target on a computer screen. Participants completed a 4-week intervention, training for 30 minutes twice per week. Training included 40 trials for each of three amplitudes trained in a session. Saccade directions were randomized and five trials were performed for each direction at a given amplitude. Saccades were recorded using the SR Eyelink-II system (SR Research Ltd., Ottawa, Ontario, Canada). Left and right eye saccades at 10°, 20°, and 30° for each participant were independently analyzed for amplitude, latency, and mean velocity of the first saccade, as well as the number of saccades to reach the target amplitude. Significance was set at p-value < 0.05. Shapiro-Wilk tests of normality of paired differences rejected normality for most tests (R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). After Bonferroni corrections, Wilcoxon Signed Rank Tests of paired differences yielded significant decreases in voluntary saccade latency in all participants at most amplitudes (among 30 amplitude comparisons, 10 were not significant). Saccade amplitude

increased significantly in one participant. After the intervention, the slope of the linear fit for main sequence plots of log of mean velocity vs log of distance decreased. The mean saccade velocity at the maximum amplitude remained the same. Thus, after the intervention the mean velocities at 10° and 20° increased towards that of the 30° amplitude. This pattern of response suggests that saccades of large amplitudes in healthy older adults respond to voluntary saccade training by lowering saccade latency and increasing mean saccade velocity at 10° and 20°.

Disclosures: P.B. Camacho: None. **R. Carbonari:** None. **C. Lopez-Ortiz:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.03/M22

Topic: E.01. Eye Movements

Support: JSPS KAKENHI Grant Numbers JP16H06752
JSPS KAKENHI Grant Numbers JP16K09709

Title: Influence of accuracy of eye movement to hand movements in visual and memorial reaching task in hereditary spinocerebellar degeneration

Authors: *S. INOMATA-TERADA[1], S.-I. TOKUSHIGE[2], S.-I. MATSUDA[3], M. HAMADA[4], S. TSUJI[4,5], Y. UGAWA[6], Y. TERAO[1];
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Abstract: Introduction: Eye and hand movements are known to be closely linked in daily actions (eye-hand coordination), for which the cerebellum is implicated not only in the motor control of individual effectors but also in their coordination. We simultaneously recorded the trajectories of the hand and eyes in two tasks: visually guided reaching task (VGR) and memory guided reaching task (MGR). **Methods:** Subjects were 12 spinocerebellar ataxia patients (SCA6 or SCA31) with pure cerebellar symptoms and 31 age-matched normal controls (NC). In VGR, a fixation spot was presented in the center of the touch panel, which the subjects gazed and touched with the tip of the index finger. After a random interval, it moved to a peripheral position, to which the subjects were instructed to move their finger by sliding on the monitor. In MGR, while subjects placed their finger at the central fixation spot, a target cue was briefly presented at a peripheral location. Subjects slid their finger toward the remembered target when the fixation spot disappeared therafter. A video-based eye tracker recorded eye movements, and the touch panel recorded the finger movements during the tasks. **Results:** Spatial distribution of trajectories of the eye and the finger was more variable in SCA patients than in NC. In VGR

there was a significant correlation between the final positions of the gaze and the accuracy of the finger in both groups (NC: r = 0.83, p = 0.001; SCA: r = 0.52, p = 0.001), but not in MGR. In both SCA patients and NC, the gaze preceded the finger to the target in VGR, whereas in MGR eye movements were often lacking or decreased. The total time from the disappearance of fixation spot to the end of the finger movement and the time of the finger sliding were significantly prolonged in SCA patients in both tasks (VGR: p = 0.04, p = 0.02 ; MGR: p = 0.002, p = 0.003), whereas the interval between the gaze and the finger was comparable to NC. The final position of the gaze did not correlate with the total time nor the finger sliding time in either groups, whereas it correlated with the interval between the gaze and the finger only in NC. **Conclusion:** Higher gaze accuracy led to higher accuracy of the finger in VGS but not in MGS, and also led the finger to follow the gaze with a shorter interval only in NC, but not in SCA patients.

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Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.04/M23

Topic: E.01. Eye Movements

Support: MOP-FDN 148418
Parkinson Canada Graduate Student Award

Title: Altered pupil dynamics in the progression of Parkinson’s disease

Authors: *J. HUANG[1], B. C. COE[1], M. SMORENBURG[1], D. BRIEN[1], D. BEATON[2], B. TAN[2], C. MARRAS[4], J. LAWRENCE-DEWAR[5], S. STROTHER[3], D. KWAN[6], P. MCLAUGHLIN[7], A. LANG[4], S. E. BLACK[8], E. FINGER[7], M. FREEDMAN[2], M. J. STRONG[9], R. SWARTZ[4], C. TARTAGLIA[4], L. ZINMAN[4], D. P. MUNOZ[1], A. THE ONDRI INVESTIGATORS[10];
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Abstract: In Parkinson’s disease (PD), the progressive loss of brain matter from neurodegeneration leads to impairments in autonomic, motor, and cognitive functions. An easy-

to-measure method that is increasingly used in clinical investigations to assess cognitive function is pupillometry. In addition to global luminance and arousal, pupil size is also modulated by converging bottom-up sensory and top-down cognitive signals. Furthermore, the circuitry for pupil control is suggested to be linked to the saccade generation system. Therefore, the disruptions in neural circuitry due to neurodegeneration should affect pupil control and its relationship to the saccade system. Here, we examined pupil dynamics in patients diagnosed with early stage PD (1~3 in Hoehn and Yahr scale) during an interleaved pro-/anti-saccade task, and hypothesized that specific components of the pupil response should be altered due to neurological deficits in PD, and these pupil measures should correspond to clinical/neuropsychological test performance. Furthermore, we examined how disease progression affect the pupil measures in annual follow-ups over 3 years. Pupil size and eye position were recorded while subjects performed the task. The pupil response following the presentation of the fixation cue consisted of an initial constriction component, which was mainly driven by the change of luminance level from fixation cue appearance, followed by a dilation component that has been previously linked to saccade suppression and voluntary saccade preparation. Analysis revealed distinct differences between PD patients and age-matched controls in pupil dynamics. Compared to controls, PD patients showed significant reduction in pupil constriction suggesting changes in the light reflex pathway, as well as reduction in pupil dilation suggesting changes in the top-down preparation signals. Neuropsychological tests and clinical scores such as the Montreal Cognitive Assessment scores and Unified Parkinson's disease rating scale correlated with pupil dilation: better test performance was associated with a stronger dilation response. Pupil measures and test performances also showed changes with disease progression. The results demonstrated changes in pupil dynamics linked to neurodegeneration in PD, showing that pupil measurements in visuomotor tasks have the potential to provide relevant early behavioural biomarker for diagnosis of neurodegenerative diseases and tracking disease progression.

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Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.05/M24

Topic: E.01. Eye Movements

Support: NIHR01NS037422

Title: Saccade vigor as an implicit measure of subjective value

Authors: *T. YOON, R. SHADMEHR;
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Abstract: Decision-making is based on the concept of utility, defined as the subjective valuation of an outcome. To measure utility, the current approach is to observe choices that subjects make. However, while choices describe ordering of preference, utility often requires measuring preference in a lottery or similar paradigms that require many observations. That is, knowing that one prefers A to B is easier to infer than assigning a numeric scale that reflects subjective value of A with respect to B. Recent work has demonstrated that if one prefers A to B, then one may move more vigorously toward A. Could vigor be a better implicit measure of utility?
We measured saccadic eye movements in humans (n=24) as they looked at visual stimuli (boxes of various color) and made choices based on the subjective value that they assigned to each stimulus. Some of the options produced a loss, while others produced a gain. Each trial began with a fixation point. On choice trials, subjects were offered a lottery: option A (100% probability of stimulus 1), vs. option B (50% probability of stimuli 2 and 3), and could choose one of them. On cued trials, only one option was provided, and were forced to choose that one. We asked whether the utility that a subject assigned to a stimulus, as measured via their decisions in choice trials, could be inferred via the vigor of their saccade toward that stimulus in the cued trials. We estimated each subject's utility for each stimulus via logistic regression with input vectors representing displayed options and output representing subject's choice.
As subjective value of a stimulus increased, for cued trials, reaction time of saccades toward that stimulus decreased, while peak velocity increased. In contrast, saccade vigor did not increase as a function of stimulus salience, which in this trial is reward size and stimulus color. Rather, vigor was lower when the saccade was toward a stimulus that promised a loss, and much lower when the loss was greater. We next looked for a causal relationship between subjective value and vigor by controlling for objective value. We found that across subjects, given a constant objective value, those who had a higher subjective value for a given stimulus also had greater relative vigor toward that stimulus.
In summary, we observed that the subjective value that people assigned to a stimulus, as measured via their choices, varied positively with the vigor (reaction time, peak velocity) of saccades that they made toward that stimulus when that stimulus was their only option.

Disclosures: **T. Yoon:** A. Employment/Salary (full or part-time):; Johns Hopkins School of Medicine. **R. Shadmehr:** 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.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.06/M25

Topic: E.01. Eye Movements

Support: NIH 1R01NS078311
ONR N00014-15-1-2312
NSF 1723967

Title: Value of error: Mechanisms that modulate sensorimotor learning

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Abstract: When we experience an error during a movement, we update our motor commands to partially correct for this error, producing adaptation. In some cases, the brain may be willing to learn more from an error if the consequence of that error is greater in one context than another. Understanding the nature of this error-dependent value function can potentially produce new ways to modulate rates of adaptation. Here, we asked whether sensorimotor learning can be accelerated through modulation of an error-dependent value function. We used saccadic adaptation as a model of sensorimotor adaptation and created a paradigm in which a decision was made based on the time available to view a stimulus. Subjects made a saccade toward a primary stimulus, but during that saccade, the primary stimulus was erased and replaced with a new, secondary stimulus, presented at a location different than the primary stimulus. This secondary stimulus was a collection of moving dots. Following completion of the primary saccade, subjects made a secondary saccade to foveate the moving dots, and then made a decision regarding motion of those dots. Therefore, in this task, the primary saccade experienced an error, which induced adaptation. That error carried a cost in terms of affecting performance on the decision-making task: the error required time to correct, thereby eliminating precious time needed to make correct decisions regarding motion of the moving dots. By varying the coherence of the motion, we varied the cost of error and then measured how this error cost affected rates of adaptation. In one environment, the decision was easy (error cost was low) and most trials were completed successfully. In the other environment, the decision was difficult (error cost was high), but performance could be improved through reduction of movement error. We found that the levels of reward experienced did not affect the rate of adaptation. That is the group that had a low error cost and acquired more successful trials, did not learn faster. Rather, the group that had a higher error cost and through adaptation could produce an increase in reward rate, learned more from error. Our results provide a new framework for understanding the extent to which the brain

is willing to learn from sensory error through mechanisms that link adaptation to changes in the reward rate.

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Poster

062. Eye Movements: Central Processing

Location: Hall A

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Program #/Poster #: 062.07/M26

Topic: E.01. Eye Movements

Support: NIH Grant R01NS078311
NIH Grant F31NS108731

Title: Signatures of the fast and slow adaptation processes during saccade adaptation

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Abstract: Behavior during motor adaptation suggests that learning may be supported by two parallel processes, one that learns significantly from error but forgets quickly (fast process), and one that learns little from error but forgets slowly (slow process). A limitation has been the inability to directly measure a behavioral proxy for each of the two hypothetical states. Here we show that, during adaptation, separate components of the commands that move the eyes during saccades carry the signatures of the putative fast and slow processes. We hypothesized that changes to the later part of the saccade would behave like the fast process, whereas changes in the early part of the movement would proceed like the slow process. To test this idea, we designed a saccade adaptation paradigm in which the target jump was perpendicular to the primary saccade direction, akin to force field adaptation where the change in motor commands is also perpendicular to the baseline commands, allowing for clear identification of the commands that result from error-dependent learning. In a blocked design (n=25), we divided each saccade into early and late halves based on peak speed and found that the motor commands during acceleration learned little from error, whereas the motor commands during deceleration responded more strongly to the same error. With passage of time, the motor commands during acceleration showed strong retention, whereas the motor commands during deceleration showed rapid forgetting. These results suggest that during saccades, the motor commands that arrive late in the movement carry a signature of the fast process whereas commands that arrive early in the movement carry a signature of the slow process. Next, we conducted a spontaneous recovery (SR) experiment in which the subject experienced a stable perturbation until reaching asymptotic learning, at which point the direction of the perturbation was switched until adaptation returned to zero; the rest of the trials were in error clamp. We performed 2 versions of the SR experiment,

one with a vertical primary saccade (n=10) and one with a horizontal primary saccade (n=10), each with perpendicular target jumps. Contrary to our expectation and the prediction of the two-state model, we found SR in the behavioral signatures of both the fast and the slow processes. Additionally, we found that the extent of adaptation was much greater for horizontal errors than for vertical errors despite the same number of trials and that this difference was specifically limited to the deceleration phase of the movement (fast process).

Disclosures: S.P. Orozco: None. **R. Shadmehr:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

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Topic: E.01. Eye Movements

Support: NIH NINDS R01NS096083
NSF CAREER SES 1352632

Title: Saccade vigor reflects preference in effort-based decisions

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Abstract: Saccade vigor has been shown to reflect how individuals evaluate rewarding financial outcomes and correlate with the value of the saccade target. However, less is known when considering costly or effortful options. In contrast to reward, the saliency (magnitude of an option's reward or punishment) and utility (option's goodness) do not go hand-in-hand. Will saccade vigor reflect the saliency of the option (i.e. greater cost) or the utility of the option (less cost)? To probe this, we provided subjects (n=22) effortful options consisting of varying inclines and durations on a treadmill while recording their eye-movements to determine the overall value of a preferred choice. Choices were between reference and alternative options of varying inclines and durations. Decisions and eye movements were recorded in two distinct phases: a deliberation phase when subjects made saccades between the two options presented on screen, followed by a decision phase where subjects confirmed their decision by making a saccade to their preferred option. An iso-cost curve was fit to each individual's choices and used to assess whether subjects based choices objectively on energetic cost using a utility model where utility was joules of energy quantified as energetic rate multiplied by duration. Results demonstrate that subjects based decisions using a subjective cost model which produced choices significantly different than otherwise predicted by an objective cost model. As cost differences increased, subjects responded more quickly. By the end of the deliberation phase, saccade vigor was greater towards

the preferred option. Saccade vigor also responded to the magnitude of differences in costs, but only when the reference was the costlier option. Subjects also fixated longer on the preferred option, and the proportion of time spent fixating increased the better that option was relative to the alternative. These results demonstrate that saccade vigor and fixations reflect the subjective utility of an option. Together these findings help us understand movement-related decisions and reveal intrinsic links between decision making and movement.

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Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.09/M28

Topic: E.01. Eye Movements

Title: The limits of saccadic frequency during visual scanning

Authors: *J. A. EDELMAN[1], A. AHMED[2];
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Abstract: While the reaction time of saccadic eye movements made to suddenly appearing visual stimuli has been studied thoroughly, much less is known about the ability to rapidly command a series of saccades irrespective of perceptual events. In this study humans made a series of six saccades across a visual target array, with all stimuli visible and stationary throughout the entire trial. Saccades were made either as rapidly as possible ("fast" condition), or, as a reference, at about one saccade per second ("slow" condition). We examined whether the maximum frequency of saccade generation in the fast condition depends upon the spatial arrangement of the visual stimuli (linear vs arranged in a square) and, to determine whether rapid fatigue occurred, the order of the saccade within the sequence of six saccades (first vs sixth). We also compared the peak velocity of saccades in the fast condition with those in the slow condition. We found a mean intersaccadic interval of ~250 ms, considerably slower than the latency of saccades to suddenly appearing targets and not considerably faster than that observed during purposive saccade scanning or reading. This was true even for subjects over-trained in a reactive saccade task, Effects of stimulus arrangement and saccade order had only modest effects on intersaccadic interval and saccade peak velocity. Saccade scanning speed (fast vs. slow) also had only a modest effect on peak velocity. These results suggest that voluntary movement initiation is a rate-limiting step in saccade scanning, thereby providing insight into neurophysiological mechanisms underlying saccadic programming and the activation of the brainstem saccade generator.

Disclosures: J.A. Edelman: None. **A. Ahmed:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.10/M29

Topic: E.01. Eye Movements

Support: NSERC Canada

Title: Gaze sampling and step location decisions during visually-guided walking with multiple foot-placement alternatives

Authors: *F. J. DOMÍNGUEZ-ZAMORA, D. S. MARIGOLD;
Dept. of Biomed. Physiol. and Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: During goal-directed motor behaviors, we often need to select a reach- or step-target location among different alternatives that can differ in their characteristics. With walking, we tend to choose stepping targets that minimize energetic cost. However, when the characteristics of the terrain in front of us become uncertain, we may be reluctant to maintain this preferred pattern. Gaze helps us to acquire environmental information to make this decision. Low uncertainty locations allow for faster information gain, and thus may drive where we direct gaze and foot placement in a continuous motor task like walking. Here we tested for the existence of a trade-off between the cost of a step and environmental uncertainty in gaze sampling and step decisions when precise foot placement was critical. We tested 5 participants on a task that required them to walk and step on four sequential targets (2D Gaussian blobs) projected on the ground. Three of the target 'rows' had one target present, and in one row, participants had a choice of two targets to step on. In this forced-choice situation, we always positioned one of these step targets at the participant's preferred step location (Low-cost target). We manipulated the uncertainty level of this Low-cost target by varying the Gaussian blob's standard deviation to create six, randomly presented uncertainty conditions. We positioned the other (High-cost) target at 4x the participant's preferred step vector. The High-cost and other targets in the sequence always had the lowest level of uncertainty. Additionally, we instructed participants to either step onto the center of each target (Precision-relevant task) or anywhere onto the targets (Precision-irrelevant task) in different blocks of trials. We used logistic regressions on the participant's choice frequencies. We found that the preference to step on the High-cost target increased with the uncertainty of the Low-cost target in the Precision-relevant ($p < 0.0001$) but not precision-irrelevant ($p = 0.132$) task. Interestingly, when the uncertainty of the Low-cost target increased, participants were more likely to direct gaze to the Low-cost target before fixating and stepping on the High-cost target in the Precision-relevant ($p = 0.0004$) but not Precision-irrelevant ($p = 0.147$) task. Therefore, we suggest that the visuomotor system is primed to prioritize stepping on and fixating targets that are associated with low motor cost. When uncertainty of the preferred

foot-placement location increases, people may visually explore both target choices to help calculate the difference between Low- and High-cost targets to generate an appropriate motor plan for the step.

Disclosures: F.J. Domínguez-Zamora: None. **D.S. Marigold:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.11/M30

Topic: E.01. Eye Movements

Support: ANR-15-CE32-0007-01

Title: Central pattern generator-driven efference copy couples eye movements with forelimb locomotion in mice

Authors: F. FRANÇA DE BARROS[1,5], C. TAILLEBUIS[1,5,6], M. MANUEL[2,5], H. BRAS[3], D. COMBES[4,6], F. M. LAMBERT[4,6], *M. BERANECK[1,5];
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Abstract: Rationale: efference copies are neural replicas of motor outputs used to anticipate the sensory consequences of a self-generated motor action. They are responsible for the coordination of motor behaviors as those intrinsic feed-forward signals generated by a local motor circuitry may influence a neural network involved in another behaviour. An example of such a predictive motor efference copy is the signal originating from the locomotor spinal CPG of Xenopus tadpoles responsible for tail undulation, which drives eye movements in order to stabilize the gaze during swimming. Here we explore whether such a predictive signal which couples eye movements to locomotion is preserved in phylogenesis, particularly in mammals. Methods and results: eye movements were studied in precollicular premammillary decerebrated adult mice. Recordings were performed headfixed and in dark while locomotion was controlled by a motorized treadmill. Locomotion epochs between 10 and 40s long and with a speed higher than 10cm/s (n=25 from 6 mice), consistently evoked eye movements mostly restricted to the horizontal plane with only minor vertical components. When present in both eyes, movements were conjugated, with an alternation of quick and slow phases of 5-10° amplitude and 1-5Hz frequency range. Forelimb activity was found to prevail over hindlimb to evoke eye movements. To determine the influence of the cervical and lumbar spinal locomotor CPGs on the eye's motor nuclei, the activity of spinal and abducens motor nerves was recorded simultaneously ex vivo in isolated brainstem-spinal cord preparations of neonatal mice (P1-P3). Rhythmic coupled

discharge was found between limb and abducens motor nerve activities during fictive locomotor bouts elicited by electrical or pharmacological stimulation. Specific activation of cervical or lumbar CPG activity revealed that cervical, but not lumbar network output is sufficient to drive the abducens nerve discharge. To study the neuronal pathway that couples the cervical CPG and the abducens nuclei, rabies virus (RV) were injected in the lateral rectus muscle of adult mice (n=5). We found infected (RV+) motoneurons (revealed by ChAT labelling) in the ipsilateral abducens nucleus as well as RV+ interneurons along the cervical spinal cord. Cervical RV+ neurons were mainly distributed ipsilaterally in the ventral horn and a few were found around the central canal. This technique revealed a dominant ipsilateral monosynaptic ascending spinal connection with abducens motoneurons. Conclusion: overall, we show that a cervical CGP-driven efference copy directly couples eye movements with forelimb movement during vigorous locomotion in mice.

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Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.12/M31

Topic: E.01. Eye Movements

Title: Human saccadic variability and perceptual judgements along the cardinal axes

Authors: *T. MALEVICH, Z. M. HAFED;
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Abstract: Saccades show notable trial-to-trial variability in their trajectories and landing positions, but they must be spatially accurate to navigate us successfully through space. Recently, in behavioral experiments in rhesus monkeys, we demonstrated that saccades are sensitive to very small tangential deviations in target position, and that observed variability in saccade trajectories might be accounted for by variability in starting eye position (due to fixational eye movements). In other words, saccade variability was in reality likely reflecting systematic corrections for variability in retinotopic target position at the time of saccade initiation. Here, we extended these experiments to human subjects, allowing us to also explore perceptual judgements of tiny tangential deviations in target position from the cardinal directions. We conducted a set of analogous experiments in naïve subjects (N = 15) combining saccadic and perceptual tasks. In a dual task, subjects had to generate a visually-guided saccade to a target located 7 deg away from display center either horizontally or vertically. The orthogonal component of target location relative to the saccade vector could be varied by +/- 0, 2.9, 5.7, or 11.4 min arc from the true cardinal axis. For example, a rightward saccade target

could be accompanied by an upward deviation of 2.9 min arc. The saccade target disappeared at saccade end, and subjects had to report whether the target appeared above (horizontal saccades) or to the right of (vertical saccades) the corresponding cardinal axis. In separate controls, the subjects simply fixated and made perceptual judgements on target location. To account for within and between subject variability, we fitted mixed effect models that included the subject and session number as random effects. Saccadic landing positions showed systematic rank ordering of direction deviation relative to orthogonal target deviation from cardinal. Time course analyses also revealed that saccade trajectories deviated to intercept the orthogonal target deviation as early as 10 ms after saccade onset. Psychometric curves for perceptual judgements (proportions of 'above' / 'right' responses) and saccadic landing positions (proportions of the landing positions being 'above' / 'to the right of' the zero offset condition landing positions) showed very strong biases along the vertical axis. These biases were more pronounced in the dual task than in the control task. Overall, our results indicate that saccades precisely re-align gaze, and with similar performance to perception; curvature of cardinal saccades likely reflects corrections due to fixational eye position variability.

Disclosures: T. Malevich: None. **Z.M. Hafed:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.13/M32

Topic: E.01. Eye Movements

Title: Eye movements during a touchscreen Archimedes spiral tracing task

Authors: *B. D. HEINTZ[1], W. E. HUDDLESTON[3], K. G. KEENAN[2];
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Abstract: Introduction. Hand function is important for everyday tasks (e.g., picking up objects, writing, opening a jar) and impairments in hand motor control are associated with decreased function and loss of independence in older adults. Archimedes spiral tracing is a common clinical assessment used in patient populations (e.g., Parkinson's disease, essential tremor) and is sensitive to age-associated changes in hand motor control. Eye movements may play a role in impaired hand motor control and age-associated changes in eye movements have been associated with increased force fluctuations during a visually guided force-matching task. However, relatively few studies have examined age-related changes in eye movements during hand motor tasks and eye movement behavior during spiral tracing is not known. Therefore, the purpose of this study is to examine eye movements during spiral tracing and to establish the feasibility of

recording eye movements during this task. **Methods.** 6 young adults (age 26-38 yrs; 3 f, 3 m) participated in this study. All participants were right handed, had normal or corrected to normal vision, and had no functional deficiencies or neuromuscular disorders. Participants sat facing an iPad mini mounted vertically to a custom-built stand. The vertical orientation of the iPad was necessary to facilitate accurate assessment of eye movements. Participants traced an Archimedes spiral displayed as a black line on the iPad touchscreen with their right index finger. Instructions were to begin in the center working outwards at a self-selected pace and to stay as close to the line as possible. Two trials were completed and total completion time was recorded. Eye movements were recorded in the horizontal (X) and vertical (Y) directions with an infrared R6 Remote Optics Eye Tracking System at 120 Hz. **Results.** All participants used saccadic eye movements during the spiral tracing task. Therefore, saccade number was calculated based on number of fixations and averaged across the two trials, defined as times when change in eye position did not exceed 0.5 degrees in the X and Y directions for a duration ≥ 100 ms. Participants made a total of 58.41 ± 13.12 saccades at a rate of 2.03 ± 0.42 saccades/s during the task. **Conclusions**. Results provide evidence for a distinct visual strategy during Archimedes spiral tracing and demonstrate the feasibility of eye movement recordings during a common, clinical measure of hand function. Future work should assess the relationship between eye movements and impaired spiral tracing performance in older adults and identify the visual strategies on other common tasks of hand function, such as the pegboard test of manual dexterity.

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Poster

062. Eye Movements: Central Processing

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Topic: E.01. Eye Movements

Support: NIH Grant EY10217
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Title: Diagonal nystagmus induced by vertical optokinetic stimulation in subjects with idiopathic infantile nystagmus

Authors: *J. R. ECONOMIDES[1], Y.-W. SUH[2], J. B. SIMMONS[1], D. L. ADAMS[1], J. C. HORTON[1];
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Abstract: In subjects with ocular misalignment and nystagmus present since early childhood, stimulation with a vertical optokinetic pattern has been reported to increase the horizontal component of the nystagmus present during fixation. The result is a diagonal movement of the eyes. We have tested subjects with either infantile nystagmus, strabismus, or both conditions to determine if this crosstalk in the optokinetic system depends on the presence of strabismus. Eye movement recordings were obtained from 9 subjects. 5 subjects had infantile nystagmus syndrome with no other associated ocular abnormalities. They exhibited typical features of congenital motor nystagmus. All had normal ocular alignment with high grade stereopsis. 1 subject had both infantile nystagmus syndrome and strabismus. 3 subjects had childhood strabismus, but no nystagmus. Subjects viewed with both eyes a noise pattern of black and white squares moving at 40°/s for 10 s while the position of each eye was monitored independently with a video eyetracker. Nystagmus traces compiled during interleaved trials of right, left, up, and down movement were compared with waveforms recorded during fixation. In all subjects, as expected, stimulation with an optokinetic pattern moving horizontally evoked nystagmus with virtually no vertical component. In the 3 subjects with childhood strabismus, stimulation with an optokinetic pattern moving vertically produced an essentially pure vertical nystagmus. In the 6 subjects with infantile nystagmus syndrome, a vertical optokinetic pattern produced nystagmus with a diagonal trajectory. It was not simply a combination of a vertical component from optokinetic stimulation and a horizontal component from the subject's congenital nystagmus. Rather, in each subject the slow phase velocity of the horizontal component during vertical optokinetic stimulation exceeded that recorded during fixation. These findings indicate that in subjects with a form of infantile nystagmus syndrome traditionally described as congenital motor nystagmus, the presentation of a vertically moving noise pattern drives a diagonal optokinetic nystagmus. This appears to arise because of crosstalk between the vertical and horizontal components of the optokinetic system. Although ocular misalignment is common in patients with congenital motor nystagmus, it is not related to the mechanism of this phenomenon, because it occurs even in the subset of individuals with this syndrome who have intact binocular function. Moreover, strabismus alone does not give rise to this phenomenon.

Disclosures: **J.R. Economides:** None. **Y. Suh:** None. **J.B. Simmons:** None. **D.L. Adams:** None. **J.C. Horton:** None.

Poster

062. Eye Movements: Central Processing

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Topic: E.01. Eye Movements

Support: NIH Grant EY027373

Title: Cortical contributions to the latency of smooth pursuit eye movements determined by simultaneous recordings in area MT and the frontal eye fields

Authors: *J. P. MAYO, S. G. LISBERGER;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Correlated features of neuronal activity constrain how the brain uses sensory information to guide behavior. While the majority of research on correlations so far has focused on the size of neuronal responses in terms of firing rate (e.g., spike count correlations), here we focus on correlations between the latency of responses between cortical neurons and their relation to the initiation of smooth pursuit eye movements. Our goal was to determine whether extrastriate visual area MT and the smooth pursuit eye movement region of the frontal eye fields (FEFsem) contribute independently or synergistically to pursuit latency. We recorded simultaneously in both cortical regions in rhesus monkeys using 24-channel Plexon V-probes. Monkeys were trained to fixate in the center of a video display and then pursue a patch of dots at various speeds, directions, and contrasts. We used a quantitative procedure that effectively shifted and scaled each trial's eye speed trace and neuronal response to obtain precise latency and amplitude estimates (Lee et al., Neuron, 2016). Consistent with previous work, we found that trial-by-trial changes in the response latencies of MT and FEFsem neurons were positively correlated with the latency of pursuit initiation. Using simultaneously recorded MT-FEFsem neuron pairs, we assessed whether fluctuations in response latencies across trials covaried between areas. The response latencies of pairs of MT-FEFsem neurons were not correlated, despite the fact that (1) latencies were correlated for pairs of neurons within each area and (2) each area's latencies were significantly correlated with the latency of behavior. These main results held for larger neuronal population sizes, where we also observed that the latency of FEFsem activity accounted for an increasingly larger portion of behavioral variance when compared to MT populations of the same size. Also, as neuronal population size increased, mixed populations of MT-FEFsem neurons continued to make more independent contributions than their MT-MT and FEFsem-FEFsem counterparts. Overall, these results suggest that MT and FEFsem make independent contributions to the timing of pursuit initiation.

Disclosures: **J.P. Mayo:** None. **S.G. Lisberger:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.16/M35

Topic: E.01. Eye Movements

Support: 5R01NS092623-03
1R01EY027373-01A1

Title: Behavioral evidence for dynamic modulation of eye velocity feedback during smooth pursuit steady-state

Authors: *S. BEHLING, S. G. LISBERGER;
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Abstract: Smooth pursuit eye movements are used by primates to track moving objects. They are initiated by a sensory estimation of target speed represented in the middle temporal (MT) area of extrastriate visual cortex and then supported by motor feedback to maintain eye speed at target speed. The motor feedback used to maintain steady-state pursuit is represented in the floccular complex of the cerebellum via Purkinje cell simple-spike activity that encodes the kinematics of the eye. Here we show that reducing the coherence in a patch of dots for a tracking target degrades eye speeds during pursuit. The deficits in pursuit are quantitatively different between steady-state pursuit and the sensory driven initiation of pursuit. Our hypothesis is that we see multiple deficits in eye speed because lowered dot coherence reduces motion reliability for pursuit initiation and separately modulates the motor signals that drive steady-state tracking. When we presented 300-ms pulses of coherence from 100% to lower values during accurate steady-state pursuit, we observed eye decelerations that were larger for lower coherences, as expected if motor feedback was reduced in gain. When we presented 300-ms pulses of target speed, we observed lower sensitivities to image motion across the retina when targets had lower values of dot coherence. However, sensitivity was not at zero, implying that disrupted sensory input cannot account for all the deficits in steady-state pursuit at low dot coherence. We have generated a simple pursuit model that uses separate modulation of visual signaling and motor feedback to account for the independent, graded effects of dot coherence on eye speed at pursuit initiation and steady-state. Our data suggest the hypothesis that reducing dot coherence creates less reliable target motion that perturbs steady-state tracking by modulation of the motor corollary discharges that comprise eye velocity memory.

Disclosures: S. Behling: None. **S.G. Lisberger:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.17/M36

Topic: E.01. Eye Movements

Support: NIH grant DC014930 (WZ)
NIH grant EY025550 (WZ)

Title: Oculomotor plant hypothesis (OPH) revisited: Abducens neuron behaviors during combined eye-head gaze shifts, disjunctive smooth pursuit and sleep in monkeys

Authors: J. HUANG[1], W. KING[2], *W. ZHOU[1];
[1]Otolaryngology and Communicative Sci., Univ. of Mississippi Med. Ctr., Jackson, MS;
[2]Otolaryngology, Univ. of Michigan, Ann Arbor, MI

Abstract: The motoneurons of the oculomotor system receive inputs from the premotor structures and send motor commands to control the extraocular muscles to generate various types of smooth and ballistic eye movements that either maintain fixation on a target against self and/or target motion (gaze stabilization) or redirect fixation to a new target (gaze shift). The relationship between motoneuron firing rate and eye movement is central to all models of gaze control. Because eye movement is generated by the summed actions of agonist-antagonist extraocular muscles, the relationship between motoneuron firing rate and eye movement should depend on how the agonist-antagonist pairs of extraocular muscles behave during an eye movement. Assuming push-pull actions of agonist-antagonist extraocular muscles, Robinson (1971) proposed the oculomotor plant hypothesis (OPH), which supposes that motoneuron activity is directly related to eye movement, no matter how the eye movement is generated. The OPH greatly simplifies neural models of gaze control and is widely accepted as the common component of current models of gaze control. Using abducens neuron firing rate-horizontal eye movement as a model, here we report that the OPH was violated in three conditions. First, when monkeys made combined eye-head gaze shifts, we found that the abducens neurons exhibited little modulation in their firing rates during the ocular counter rotation (OCR) phase of combined eye-head gaze shifts. Second, when monkeys tracked a target moving along the visual axis of an eye, the monkeys made disjunctive smooth eye movement. We found that their firing rates were substantially modulated when there was no movement in the eye they innervated. Third, when monkeys were in sleep, they exhibited slow drifting eye movements that were often disjunctive. We found that the relationships between abducens firing rate and eye position/velocity were substantially different from that during awake. These results suggest that the OPH, which is valid during single type of eye movement, may not be appropriate during conditions that involve multiple eye movements. These results put important constraints on new models of gaze control in natural conditions.

Disclosures: J. Huang: None. **W. King:** None. **W. Zhou:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.18/M37

Topic: D.08. Visual Sensory-motor Processing

Support: IBS-R015-D1

Title: The interaction between prior knowledge and sensory evidence revealed in multivariate EEG activity pattern during smooth pursuit eye movement

Authors: *W. JEONG[1], J. LEE[2], S. KIM[3];
[1]Sungkyunkwan Univ., Suwon Si, Gyeonggi-Do, Korea, Republic of; [2]Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon-Si, Gyeonggi-Do, Korea, Republic of; [3]Sungkyunkwan Univ., Gyeonggi-Do, Korea, Republic of

Abstract: Whenever we make actions or reactions, our brain combines incoming sensory evidence and prior knowledge to make optimal behavioral responses. Earlier studies have shown that the influence of prior knowledge on our behavior becomes more prominent when sensory evidence is ambiguous or weak. Probing the inter-relation between prior knowledge and incoming sensory evidence with corresponding neural activity is important to understand the neural basis of human sensory-motor behaviors. To understand the neural mechanisms underlying the integration of prior knowledge and sensory evidence for sensory-motor behaviors in human, we asked human participants to track visual motion stimuli that randomly moved in one of the three directions (pre-determined central direction, +30, -30 deg) while we collected EEG activity using a 64-channel active electrode system (BrainAmp, Brain Products, GmbH). We controlled the strength of the sensory evidence by randomly varying the luminance contrast of the pursuit target (100% or 12%). Prior knowledge for motion direction was manipulated by a motion cue that was presented before the pursuit target and we controlled the validity of the motion cue to measure the influence of the prior knowledge on behavior. Comparison of eye velocity direction traces in two outer motion directions among the three (central direction, +30, -30 deg) revealed the clear influence of the cue-induced prior knowledge on the initiation of smooth pursuit eye movements, especially when the contrast of the pursuit target is 12%. Timing of the significant difference between the two pursuit direction traces was delayed when the direction of the cue did not match with that of the target. Interestingly, when we measured the differences in multivariate EEG activity patterns using Mahalanobis distances (measured in 47-channel space), the timing of the significant distance also gets delayed in 12% contrast condition. The changes in neural state-space happen in advance of behavioral changes. Our preliminary results suggest that the dynamics of interaction between prior knowledge and incoming sensory evidence can be predicted by distances in the neural state-space of multivariate EEG activity.

Disclosures: W. Jeong: None. **J. Lee:** None. **S. Kim:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.19/M38

Topic: E.01. Eye Movements

Support: NIH Grant TL1 TR 001871
NIH Grant R41 NS100222-01A1
That Man May See

Title: Fixational eye motion characteristics in a large, healthy control population as measured by the tracking scanning laser ophthalmoscope

Authors: *C. K. SHEEHY[1], E. BENSINGER[2], A. J. GREEN[1];
[1]Neurol., UCSF, San Francisco, CA; [2]Vision Sci., UC Berkeley Sch. of Optometry, Berkeley, CA

Abstract: The human eye is constantly in motion during fixation. However, most fixational eye motion studies to date have focused on smaller and/or trained cohorts of healthy controls without considering the impact of age on fixation. In this study, we aimed to discover if monitoring involuntary fixation could give micron-level motor function insight into the aging brain. We recorded both microsaccade and drift characteristics in a control population ranging in age from 18-89 years utilizing a custom built, retinal eye-tracking device - the tracking scanning laser ophthalmoscope (TSLO). We recruited 100 controls without any history of neurological or retinal disease. High-resolution retinal eye-tracking was performed using 840 nm light to raster scan the retina. A chin rest with temple pads was used to limit head motion. Three, 10-second recordings of fixation per eye, spanning a 5° field of view, were acquired monocularly for each subject. Participants were instructed to fixate on the upper right-hand corner of the imaging raster. Strip-based, offline analysis of the retinal images was used to extract eye motion at 480 Hz. Microsaccade velocity, acceleration, amplitude, and number of microsaccades were calculated, along with drift metrics of amplitude and speed. Participants were placed into two age groups for analysis: $\leq$ 50 years (N=51) or > 50 years (N=49) which totaled 4,779 microsaccades and interspersed drift epochs. We found that the average number of microsaccades in a 10 second recording was 9.8 (SD 4.6) for controls $\leq$ age 50 and 14.1 (SD 6.7) for those > age 50. Additionally, the (1) mean amplitude (CI) [21.5 arcmin (20.7-22.3) vs. 27.4 arcmin (26.5-28.3); t (4777) = -9.6, $p<0.001$], dominated by the horizontal contribution of amplitude [18.6 arcmin (17.8-19.4) vs. 25.3 arcmin (24.4-26.2); t (4777) = -10.68, $p<0.001$], the (2) mean velocity [45.3 °/s (44.0-46.6) vs. 53.1 °/s (51.9-54.3); t (4777) = -8.6, $p<0.001$], and the (3) mean acceleration [14,707.9 °/s^2 (14,182.1-15,233.7) vs. 19,291.8 °/s^2 (18,806.8-19776.8); t (4777) = -12.5, $p<0.001$] were all significantly different between the $\leq$ 50 and the >50 control groups, respectively. The mean drift speed for each drift segment was minimally larger in the >50 control group [1.40 °/s (1.39-1.42) vs. 1.46°/s (1.44-1.49); t(1357)=-4.92, $p<0.001$)]. Drift amplitude between the two groups showed no statistical difference. This data suggests that increasing age could play a role in microsaccade amplitude, velocity, and acceleration, with a minimal role in drift characteristics, and should be accounted for when comparing to neurological or retinal disease patients.

Disclosures: C.K. Sheehy: A. Employment/Salary (full or part-time):; UCSF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C. Light Technologies, Inc., UCSF. **E. Bensinger:** A. Employment/Salary (full or part-time):; UC Berkeley. F. Consulting Fees (e.g., advisory boards);

C. Light Technologies. **A.J. Green:** A. Employment/Salary (full or part-time):; UCSF. 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.; Novartis. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UCSF. F. Consulting Fees (e.g., advisory boards); Bionure, Medimmune, Mylan Pharmaceuticals, Amneal Pharmaceuticals, Inception Sciences.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.20/M39

Topic: E.01. Eye Movements

Support: JSPS Grant-in-Aid for Scientific Research (B) 16H02901

Title: Predictive accommodation control in humans

Authors: S. UMEMOTO[1], *Y. HIRATA[2];
[1]Computer Sci., [2]Robotic Sci. and Technol., Chubu Univ. Col. of Engin., Kasugai, Japan

Abstract: When we shift our gaze, not only are the eyes and head directed to an object of interest, but the eyes' lenses are adjusted to get a focused image of the object on the retina through a process called accommodation. It has been known that predictive eye movements are acquired in goldfish (Miki et al., J. Neurosci, 2018) and in humans (Matsuzawa et al., Soc. Neurosci. Abstr, 2017) when exposed to a repetitive periodic visual motion stimulation. Namely, the eyes start to accelerate or decelerate prior to the initiation or termination, respectively, of an abrupt visual motion stimulus. By contrast, it has not been well known whether the accommodation system is also capable of such predictive control. If it does not work predictively, similarly to the oculomotor system, the quality of retinal images after gaze shifts would be degraded even when the eyes are directed predictively to an object of interest. Here we examined the predictive ability of the accommodation system in humans. Our subjects (N=6, male university students, 21-24 yo) were instructed to follow a moving visual target through an auto kerato-refractometer (Shigiya Machinery Works LTD, WAM-5500) while monocular (right eye) accommodation [D] was recorded at a sampling rate of 6 Hz. The visual target was moved sinusoidally at a frequency of 0.25, 0.5, 0.75, or 1.0 Hz in front of the subjects along the visual axis of the measured eye, while the unmeasured (left) eye was occluded. We found that phase lag for the accommodation response was shortened from the second cycle of the sinusoidal target motions. Also, accommodation response gains (accommodation amplitude / stimulus amplitude) increased temporarily after a few cycles of the visual stimulation. Furthermore, when the visual target was extinguished suddenly, accommodative response continued as if following the

invisible visual target. These results suggest that the accommodation system is also under adaptive predictive control that may effectively work in tandem with the oculomotor system.

Disclosures: Y. Hirata: None. **S. Umemoto:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.21/M40

Topic: E.01. Eye Movements

Support: USPHS EY008313
Researchto Prevent Blindness

Title: Eye size and the kinematics of horizontal eye rotation

Authors: *J. L. DEMER[1], R. A. CLARK[2];
[1]Ophthalmology & Neurol., [2]Ophthalmology, UCLA, Los Angeles, CA

Abstract: The eye does not rotate about a fixed center, but translates as it rotates horizontally in a rolling motion. Determination of oculorotary torques requires consideration of both tensions and lever arms of extraocular muscles, but lever arms could depend upon the size of the eyeball. Depending on refractive error, globe axial length (cornea to retina distance) can vary considerably. We used magnetic resonance imaging (MRI) to evaluate the effect of axial length on rotational axes and lever arms of horizontal rectus muscles.
In 57 adult humans (68 eyes) having a wide range of eye sizes, 390-micron resolution surface coil, T2 fast spin echo MRI was repeated in 2 mm thick axial planes in target-controlled central gaze, large abduction, and large adduction. Globe axial lengths were measured from images containing the largest globe cross-sections. Globe centers were calculated from area centroids of the largest globe cross-sections omitting corneas. Displacements of lens centers and globe-optic nerve junctions in eccentric gaze were used to calculate axes of rotation in orbital coordinates. Lever arms were calculated as the distances between muscle insertions and rotation axes. Mean abduction was 29±5.8° (standard deviation), and adduction was 34.2±8.1°. Average globe rotational axis for abduction was 0.5±2.1mm lateral and 0.5±2.1mm anterior to initial geometric globe center, and for adduction was 0.7±2.2mm medial and 2.0±4.2 mm anterior. Lever arms for abduction (relative to initial globe position) were 12.3±3.5mm for the medial rectus (MR) and 11.8±2.6mm for the lateral rectus (LR) muscle. Lever arms for adduction were 8.5±3.3mm for MR and 12.6±2.7mm for LR. Rotational axes did not vary significantly with axial length. However, because scleral insertion locations are uniform with respect to the corneal limbus, with the exception of the MR in adduction, the lever arms did vary significantly with axial length ($P<0.01$). On average, the LR lever arm increased by 60±10% of axial length for abduction and

35±13% for adduction, while the MR lever arm increased by 60±12% of axial length for adduction.
The complex kinematics of eye movement would be expected to be reflected in the motor commands generated by the ocular motor control system. This behavior is likely to vary with eye size, albeit not in direct proportion to the axial length of the eye.

Disclosures: J.L. Demer: None. **R.A. Clark:** None.

Poster

062. Eye Movements: Central Processing

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 062.22/M41

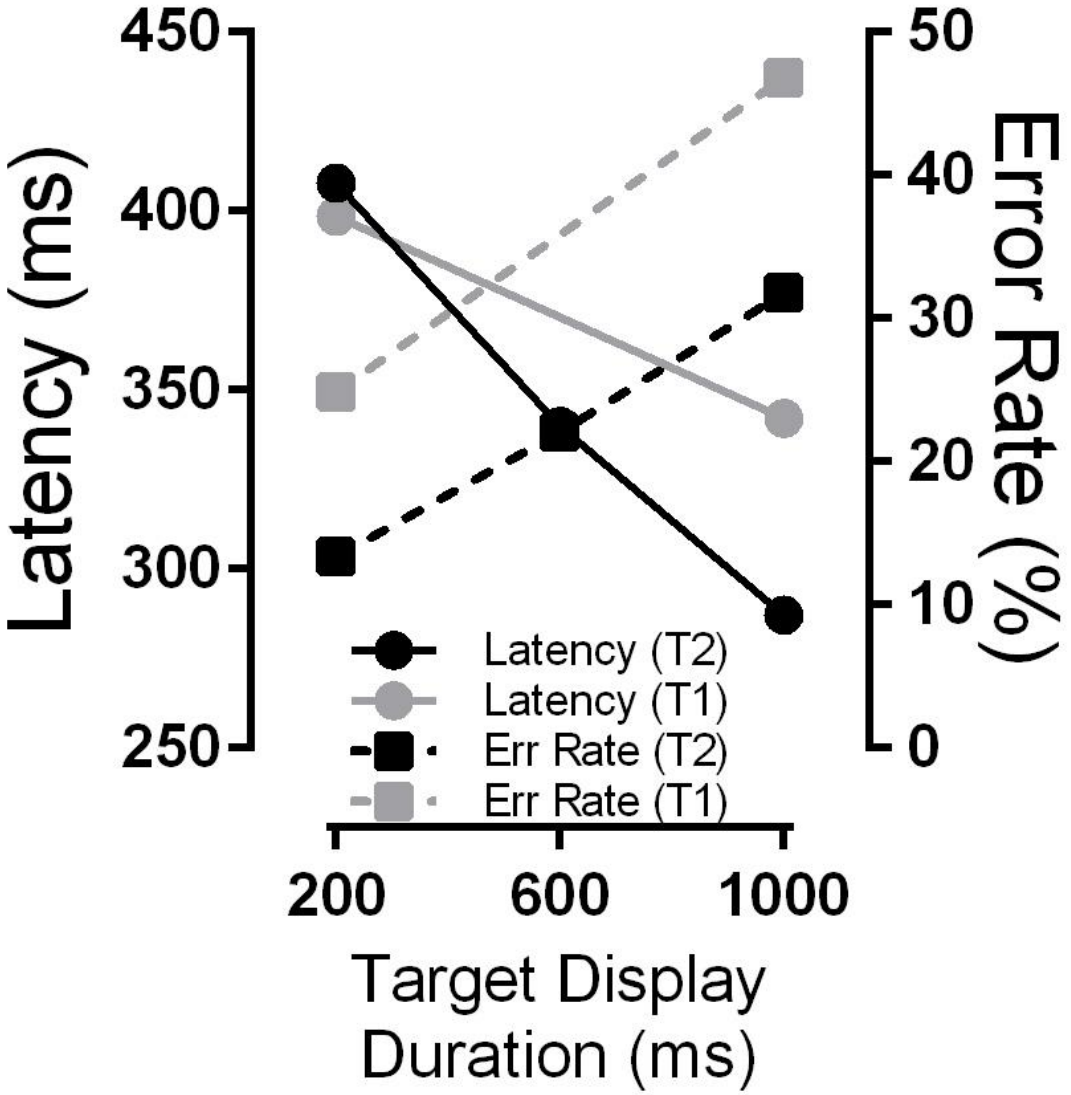
Topic: E.01. Eye Movements

Title: Optimising the minimally delayed oculomotor response task: The effect of decreased spatial and increased temporal uncertainty

Authors: *P. C. KNOX[1], D. LIANG[2];
[1]Eye & VIsion Sci., Univ. of Liverpool, Liverpool, United Kingdom; [2]Sch. of Physical Educ. and Sports Sci., South China Normal Univ., University Town, China

Abstract: The minimally delayed oculomotor response task (MDOR; participants inhibit saccades to target onsets and instead saccade to target offsets; Knox et al 2018, Exp Brain Res 236:2867) provides an oculomotor means of measuring behavioural inhibitory control. Normal ageing affects performance on this task, and error rates can be very high in older participants. We investigated the addition of target placeholders (reducing spatial uncertainty) and using 3 rather that 2 target display durations (increasing temporal uncertainty).A group of 13 healthy, adults (mean age: 44y; range 25y-71y) were tested with the new version of the MDOR task (T2), of whom 9 had been tested on the previous version (T1). A central fixation target and two square saccade target placeholders (5° left/right of fixation) appeared at the start of each trial. After a randomised fixation period (1-1.5s), the central fixation target was extinguished and a target appeared in one of the placeholders for a display duration (DD) of 200ms, 600ms or 1000ms (DD and direction randomised). Participants were insructed to maintain fixation centrally and saccade to the target position (the centre of the target placeholder) on target offset. Eye movements were recorded using an infrared eye tracker; latency and amplitude of target directed primary saccades were measured. Saccades occurring <80ms post target offset were classed as errors.In T2, saccade latency and error rate correlated with DD (N=13; latency: Spearman's rho=-0.59, $p<0.001$; ER: 0.51, $p=0.001$). In the 9 participants who completed both T1 and T2, error rates were consistently lower in T2 for all DDs by approximately 10% (see Fig). When tested with ANOVA, both task (T1 vs T2) and DD (200ms vs 1000ms), were significant ($p=0.007$ and $p<0.001$ respectively). Latency, while more variable, was lower for 600ms and

1000ms DDs, with a larger overall modulation in T2. While older participants again had higher error rates and longer latencies, the modified MDOR task may be useful in avoiding error rate ceiling effects.



Disclosures: **P.C. Knox:** None. **D. Liang:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.01/M42

Topic: E.02. Cerebellum

Support: ANR-14-CE17-0006
ANR-18-CE19-0024-02
ANR-11-LABX-0015

Title: Metabotropic dependent and independent supralinear calcium signals associated with paired parallel fibre and climbing fibre stimulation in cerebellar purkinje neurons

Authors: *M. CANEPARI, K. AIT OUARES;
LIPhy, CNRS UMR 5588, St Martin D'Heres, France

Abstract: In cerebellar Purkinje neurons (PNs), the concomitant activation of parallel fibre (PF) climbing fibre (CF) inputs underlie a Ca^{2+} signal larger than the linear summation of the two synaptic pathways alone (supralinear Ca^{2+} signal) in the dendritic site of PF inputs. Imaging Ca^{2+} transients at 5 kHz with the low-affinity indicator Oregon Green BAPTA-5N in PNs of the mouse, we found that supralinear Ca^{2+} signals occur when a CF excitatory postsynaptic potential (EPSP) follows a train of 5 PF-EPSPs at 100 Hz. However, when the CF-ESPS is delayed by 60 ms from the first PF-EPSP, the supralinear Ca^{2+} signal is unaffected by the mGluR1 antagonist CPCCOEt. In contrast, when the CF-ESPS is delayed by 100-150 ms from the first PF-EPSP, the supralinear Ca^{2+} signal is inhibited by CPCCOEt and occurs at the peak of the slow mGluR1-dependent EPSP. Using membrane potential imaging and Ca^{2+} imaging with the high-affinity indicator Fura2 we demonstrate that the mGluR1-independent supralinear Ca^{2+} signal is due to two mechanisms: 1) boosted Ca^{2+} influx via P/Q-type voltage-gated Ca^{2+} channels due to the inactivation of A-type K^+ channels produced by dendritic depolarization; 2) transient saturation of endogenous Ca^{2+} buffers produced by preceding Ca^{2+} influx during PF-EPSPs. None of these two mechanisms is involved in the mGluR1-dependent supralinear Ca^{2+} signal which is also not mediated by Ca^{2+} release from stores. In contrast, the mGluR1-dependent supralinear Ca^{2+} signal is inhibited by the channel blocker IEM1460 that also inhibits the slow mGluR1-dependent EPSP and the two phenomena are linearly correlated. Thus, we suggest that the non selective cation conductance underlying the slow mGluR1 EPSP is implicated in the the mGluR1-dependent supralinear Ca^{2+} signal. These results shed new light on one of the most important phenomenon in cerebellar physiology.

Disclosures: M. Canepari: None. **K. Ait Ouares:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.02/M43

Topic: E.02. Cerebellum

Support: NIH Grant 1P50NS098685.
NIH/ORIP Grant P51OD011131

Title: Structural and ultrastructural plasticity of Purkinje neurons and glutamatergic climbing fibers in the cerebellum motor areas of MPTP-treated Parkinsonian monkeys: A 3D quantitative analysis

Authors: *R. M. VILLALBA[1], B. CHANEY[1,2], S. WEGEMER[1,2], R. JANG[1], Y. SMITH[1,3], J.-F. PARE[1];
[1]Yerkes Resch Ctr. and Udall Ctr. of Excelence For Parkinson's Disease, Emory Un, Decatur, GA; [2]Furman Univ., Greenville, SC; [3]Dept. of Neurology-School of Medicine, Emory Univ., Decatur, GA

Abstract: The cerebellum controls the coordination of voluntary movement, gait, posture and motor functions. Functional neuroimaging data in Parkinson’s disease (PD) patients have demonstrated cerebellar atrophy related to both increases and decreases in cerebello-cortical connectivity. Although such findings suggest cerebellar involvement in PD, neuroanatomical studies are needed to elucidate cerebellar pathology. In this study, cerebellar sections from 3 control and 3 MPTP-treated parkinsonian monkeys have been used to assess changes in the cerebellum volume and the number of Purkinje (PK) neurons, and to obtain 3D models of PK cell dendrites and their climbing fibers (CFs) inputs. Stereological analysis of calbindin-immunostained (immunoperoxidase) sections showed that the volume of the cerebellar cortical areas receiving motor inputs is reduced by 35%, and the number of PK neurons in this region decreases by 28% in parkinsonian monkeys compared with controls. 3D light microscopy analysis of confocal images from cerebellar cortex sections double immunostained (immunofluorescence) for calbindin and vGluT2 (specific marker for CFs) revealed: 1.- The total length of the individual dendritic trees of PK neurons is reduced ~40% in parkinsonian animals. 2.- The density of vGluT2-positive contacts on both the proximal and distal dendrites of PK neurons is reduced by 40-65% in parkinsonian monkeys. For the 3D-ultrastructural approach, serial electron microscopy images (single block face scanning electron microscopy) obtained from coronal sections (1 control, 1 MPTP-treated monkey) of cerebellar cortex immunostained for vGluT2 have been used. The quantitative analysis of the 3D-reconstructed glutamatergic synapses has revealed that the volume of the vGluT2 terminals is reduced by ~50% in the parkinsonian monkey. Comparative analysis of the size of postsynaptic densities and volume of

the dendritic spines of PK neurons are in progress. Our data suggest an atrophy of the cerebellum motor areas and a pathological alteration in the intrinsic cerebellar connectivity in parkinsonian monkeys. Such a change could affect the inhibition of PK neurons upon deep cerebellar nuclei, and the organization of the connectivity between the cerebellum and large-scale cortical networks in parkinsonism, thereby contribute to some of the PD motor (and non-motor) symptoms.

Disclosures: R.M. Villalba: None. **B. Chaney:** None. **S. Wegemer:** None. **R. Jang:** None. **Y. Smith:** None. **J. Pare:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.03/M44

Topic: E.02. Cerebellum

Support: NIH Grant NS092623

Title: Transfer of cerebellar motor learning in smooth pursuit eye movements between sites with distinct behavioral and neural properties

Authors: *D. J. HERZFELD, S. G. LISBERGER;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: We have constrained the site and neural mechanisms for the acquisition and expression of cerebellar-dependent motor learning through behavioral experiments that strategically manipulated the conditions used to cause learning and probe motor memory. Rhesus monkeys tracked a smoothly moving target that began to move with a predefined “pursuit” speed. An instruction for learning occurred 250ms later when target direction changed due to the addition of an orthogonal velocity component. We probed the motor memory via pursuit targets that moved at a constant velocity, revealing well-timed learned eye movements that anticipated the change in the target’s direction, even after the presentation of a single instructive stimulus; the size of the memory grew across repetitions of the instruction. Generalization of memory, assayed via probe trials that moved with a different pursuit speed than the instructive trial, changed dramatically across the course of learning. After learning with one or a few consecutive instructive trials, the motor memory scaled linearly with pursuit speed. Gradually, over 1,000 trials, the pattern of generalization changed qualitatively. We conclude that the initial motor memory is gradually transferred to a second site where the properties of the signals that are subject to learning also different qualitatively. We were able to constrain the neural signals that are subject to learning at these two sites of plasticity through a computational model of pursuit learning that includes a potential mechanism for the transfer of learning across sites. Our data

and model suggest multiple learning processes with distinct effects on the overall motor memory over diverse time courses, likely arising from two different sites of plasticity.

Disclosures: D.J. Herzfeld: None. **S.G. Lisberger:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.04/N1

Topic: E.02. Cerebellum

Support: hfsp
isf
erc

Title: Cerebellar climbing fiber coding of eye movements and reward expectation in monkeys

Authors: *N. LARRY, M. YARKONI, A. LIXENBERG, M. JOSHUA;
The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The cerebellum has been hypothesized to perform supervised motor learning. In this framework, climbing fiber inputs to the cerebellum results in unique events termed complex spikes and encode error signals that instruct learning. Recently, evidence has accumulated to suggest that the cerebellum is also involved in the processing of reward. To study how rewarding events are encoded, we recorded the activity of climbing fibers when monkeys were engaged in an eye movement task. At the beginning of each trial, the monkeys were cued to the size of the reward that would be delivered upon successful completion of the trial.
We found that climbing fiber activity differentiated between different reward sizes during cue presentation when information about reward size was first made available. Specifically, the complex spike rate was higher when the expected reward size was large. Future reward size did not modulate activity at reward delivery or during eye movements. In agreement with the error signal model, complex spike and simple spike rate were oppositely modulated during movement. However, we did not find a similar modulation during cue presentation. This implies that the complex spike reward and error signals operate through different mechanisms.
These results indicate that climbing fibers encode the expected reward size and suggest a general role of the cerebellum in associative learning beyond error correction. Our results are consistent with a time difference learning model in which climbing fiber activity represents reward prediction error, similarly previous work on dopaminergic neurons. The model has strong predictions on complex spike activity when a reward is given unexpectedly; and when a reward is predicted with high certainty but omitted. As a first step towards testing this prediction, we have designed a probabilistic task in which we manipulated the reward expectancy both at the

cue and the time of reward delivery. We found that smooth pursuit behavior was sensitive to this probability as monkeys tracked more accurately and selected the targets that lead to higher probabilities of reward. This task will allow us to probe the interactions between reward prediction signals and cerebellar representation of behavior.

Disclosures: **N. Larry:** None. **M. Yarkoni:** None. **A. Lixenberg:** None. **M. Joshua:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.05/N2

Topic: E.02. Cerebellum

Support: NIH Grant 5-R01-NS078311
the Office of Naval Research (N00014-15-1-2312)
the National Science Foundation (CNS- 1714623)

Title: Sensory prediction error, not motor error, drives complex spikes in the Purkinje cells of cerebellum

Authors: *K. KARBASI[1], D. J. HERZFELD[2], Y. KOJIMA[3], R. SOETEDJO[3], R. SHADMEHR[1];
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Abstract: In the oculomotor vermis (OMV) region of the cerebellum, Purkinje cells (P-cells) produce simple spikes that transform efference copy into predictions about kinematics of the ongoing saccadic eye movement. If the movement ended in error, the P-cell is likely to produce a complex spike (CS), and the probability of the CS depends on the direction of the movement error: each P-cell has a preferred direction of movement error for which it has the highest probability of CS. What is the origin of this CS tuning? Here, we hypothesized that the CS tuning to motor error arises from the inputs that the inferior olive receives from the superior colliculus. In this framework, when a movement ends in error, the stimulus is not on the fovea, but rather in a peripheral region which results in excitation of congruent sites in the colliculus and production of a corrective saccade. If the source of the complex spikes is collicular activity, then P-cells should produce complex spikes not just because of movement errors, but also because of any visual event that produced activity on the colliculus. According to this framework, P-cells should produce a complex spike with similar tuning properties both in response to presentation of a visual stimulus that results in a primary saccade, as well as a visual stimulus that produces a corrective saccade. Notably, the directional tuning properties of the complex spikes should be the same in both conditions. Considering that there exists significant

projections from the colliculus to the inferior olive, and that the colliculus encodes a retinotopic map of the visual field, we predicted that any stimulus-induced activity in the colliculus should affect the CS probability in the P-cells. That is, the probability of CS firing should be modulated, with or without an accompanying motor error, by the relative location of a salient stimulus in the periphery of the fixation point. We recorded from the OMV of the macaque while the animals performed voluntary saccades. We found that whether the experienced error was due to an induced stimulus perturbation or originated from internal motor variability, the P-cells encoded the direction of that error via a similar CS-tuning curve. Furthermore, we found that the CS-tuning was consistent regardless of whether a visual stimulus was presented and resulted in a primary saccade, or that the primary saccade ended with a motor error. It appears that the CS tuning of P-cells in classic saccadic adaptation experimental paradigms is not due to the perceived motor error, but a result of the stimulus-induced activity in the colliculus, corresponding to the vector that originates from the eye location to the stimulus location in the periphery.

Disclosures: K. Karbasi: None. **D.J. Herzfeld:** None. **Y. Kojima:** None. **R. Soetedjo:** None. **R. Shadmehr:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.06/N3

Topic: E.02. Cerebellum

Support: R01NS073919
R01NS098308

Title: Sensory modulation of climbing fiber bursts in the cerebellum of awake mice

Authors: A. S. FANNING[1], R. A. CHITWOOD[2], J. J. SIEGEL[2], M. D. MAUK[1], *H. NISHIYAMA[1];
[1]Univ. Texas, Austin, Austin, TX; [2]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Climbing fibers (CFs) provide strong excitatory inputs to cerebellar Purkinje cells and contribute to motor control and motor learning. They discharge approximately once per second, and each event consists of a high-frequency (200-500 Hz) burst of several action potentials. While the low-frequency CF discharges have been studied extensively, the pattern of high-frequency bursts and its functional significance in awake animals are largely unknown. Here, we imaged axonal calcium transients in CFs and found that the occurrence and strength of sensory stimuli enhanced the magnitude of burst-evoked calcium transients in awake, behaving mice. Furthermore, the magnitude of CF calcium transients gradually increased or decreased depending

on the context of long-term sensory experiences. Since the magnitude of CF calcium transients is scaled with the number and interspike interval of action potentials in a burst, these results indicate a rich repertoire of burst modulation, which significantly expands the coding capability of individual CFs.

Disclosures: A.S. Fanning: None. **R.A. Chitwood:** None. **J.J. Siegel:** None. **M.D. Mauk:** None. **H. Nishiyama:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.07/N4

Topic: E.02. Cerebellum

Support: CAS Grant QYZDB-SSW-SMC032
NSFC Grant 31722025

Title: Cerebellar climbing fibers convey reward signals during motor learning in monkeys

Authors: H. LIU, Y. HU, ***Y. YANG**;
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Abstract: Seeking reward is a positive motivational factor to optimize motor skill learning. Recently, researches shine a light on that cerebellum could involve in processing of reward. To test how reward may impact cerebellar plasticity and motor learning, we recorded the single unit activity of Purkinje cells in the cerebellar floccular complex of a rhesus monkey as he tracked in smooth pursuit directional learning task (Medina and Lisberger, 2008, Yang and Lisberger, 2014). First, we assessed the direction selectivity of simple spike firing of a PC and customized “ON” and “OFF” instructive directions. During learning, these two opposite instructive changes in target motion will be randomly introduced. Once monkey successfully completed a trial, one of two sizes of reward were delivered with a fixed association with that instruction. For example, if a bigger size reward (0.15ml)was given in “OFF” learning trials, a smaller size reward (0.05ml) would be delivered in “ON” learning trials. The rule of reward was changed in different learning blocks. Our data showed that the timepoint of complex spikes of PCs to instructions was shifted with reward signals. Interestingly, there is an increasing CS activation even before instruction onsets during reward associated motor learning. These results suggested that the activation of climbing fibers can convey reward signals during motor learning.

Disclosures: Y. Yang: None. **H. Liu:** None. **Y. Hu:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.08/N5

Topic: E.02. Cerebellum

Support: NIH Grant NS072406

Title: Enhanced oculomotor learning in mice with impaired LTD

Authors: *A. M. SHAKHAWAT, J. N. BHATEJA, M. GAGNON, J. RAYMOND;
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Abstract: Long-term depression (LTD) at the parallel fiber-Purkinje cell synapses has been proposed as a plasticity mechanism supporting cerebellum-dependent motor learning. However, the effects of experimental manipulations of LTD on behavior have been variable, with LTD-impaired mice exhibiting either impaired or normal cerebellum-dependent learning. Moreover, sometimes a perturbation of LTD can have different effects on different cerebellum-dependent learning tasks. Here we report a selective enhancement of a certain form of cerebellum-dependent learning in mice with impaired LTD.

We measured oculomotor learning in two lines of mice with impaired LTD, GluR2Δ7 KI and GluR2K882A KI. Depending on the stimulation protocol used for LTD induction, mild to severe impairment of LTD at the parallel fiber-Purkinje cell synapses of these mice was observed (Yamaguchi et al 2016), yet vestibulo-ocular reflex (VOR) learning was found to be normal (Schonewille et al, 2011). However, previous work has demonstrated that the presence or absence of a VOR learning phenotype is sensitive to the parameters of training (Boyden et al, 2006; Nguyen-Vu et al, 2016), therefore we tested VOR learning in GluR2Δ7 KI and GluR2K882A KI using an extended range of training conditions. Surprisingly, we found a significant enhancement rather than impairment of VOR learning in these LTD-impaired mouse lines. The learning enhancement was selective for learning to increase the VOR gain in response to high frequency (1 Hz) visual-vestibular training. Learning to increase the VOR gain in response to low frequency (0.5 Hz) visual-vestibular training was not significantly altered, nor was learning to decrease the VOR gain in response to high or low frequency training.

The selective effects of abnormal LTD on high-frequency VOR-increase learning are consistent with previous studies. However, the enhancement of this form of VOR learning with chronic impairment of LTD stands in contrast to the recent finding of impaired high-frequency VOR-increase learning with acute impairment of LTD (Kakegawa et al., 2018). We will discuss the implications of these results for understanding how LTD functions in the intact circuit over time.

Disclosures: A.M. Shakhawat: None. **J.N. Bhateja:** None. **M. Gagnon:** None. **J. Raymond:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.09/N6

Topic: E.02. Cerebellum

Support: NIH-NS072406
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Simons Foundation-543031

Title: Experience tunes the timing requirements for synaptic plasticity in the cerebellum

Authors: *S. JAYABAL[1], A. SUVRATHAN[2,1], J. DISANTO[1], J. L. RAYMOND[1];
[1]Dept. of Neurobio., Stanford Univ. Sch. of Med., Stanford, CA; [2]Neurol. and Neurosurgery, Pediatrics, McGill Univ., Montreal, QC, Canada

Abstract: Recently, we discovered a striking heterogeneity in the rules governing associative plasticity, with different timing rules for the induction of associative plasticity at different parallel fiber-Purkinje cell synapses in the cerebellum. Here we report evidence that this heterogeneity arises through experience and a novel form of metaplasticity. In particular, we show that the timing rules for the induction of associative LTD are themselves plastic, and that this metaplasticity tunes the LTD to the feedback delay in the circuit.
In the cerebellar flocculus *in vivo*, the feedback delay between the parallel fiber activity generating an eye movement and the climbing fiber activity signaling an oculomotor error is ~120 ms. In slices of the flocculus, LTD is selectively induced by the same, 120 ms parallel fiber-climbing fiber pairing interval. This precise matching of the timing rules for LTD to the feedback delay in the circuit should ensure that LTD is selectively induced in the parallel fiber synapses that contributed to an error. We tested whether this precise matching of the properties of LTD to the circuit properties depends on experience.
To eliminate experience of the 120 ms feedback delay in the flocculus, we eliminated the visual error signals carried by the climbing fibers, by dark-rearing C57Bl/6 mice from birth. This perturbation of visual experience altered the timing rules for induction of LTD in the flocculus. In dark reared mice, a 120-ms parallel fiber-climbing fiber pairing interval failed to induce LTD, in contrast to normal-reared mice, which had experienced this feedback delay throughout development. Moreover, a 0-ms pairing interval (coincident parallel fiber and climbing fiber activation), which does not induce LTD in normal-reared mice, was effective at inducing LTD in the dark-reared mice. Thus, the timing rules for associative plasticity in the cerebellum are metaplastic, and are tuned to the feedback-delay in the circuit through experience.

Disclosures: S. Jayabal: None. **A. Suvrathan:** None. **J. Disanto:** None. **J.L. Raymond:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.10/N7

Topic: E.02. Cerebellum

Support: NS105958

Title: A disinhibitory microcircuit to gate climbing fiber-mediated learning

Authors: *K. ZHANG[1,3], A. BONNAN[4], G. G. GROSS[5], D. B. ARNOLD[6], J. M. CHRISTIE[2]; [2]Synapse Physiol. Group, [1]Max Planck Florida Inst., Jupiter, FL; [3]Florida Atlantic Univ., Jupiter, FL; [4]MPFI, Jupiter, FL; [5]Mol. Biol., [6]USC, Los Angeles, CA

Abstract: Inhibition from molecular layer interneurons (MLIs) is sufficient to override the ability of climbing fibers to instruct learning during adaptation of the vestibular-ocular reflex (VOR) by suppressing the Purkinje cell dendritic Ca^{2+} response to climbing fiber excitation. However, to assess for the necessity of MLIs in gating climbing fiber signaling during behavior, we used a genetically encoded toolkit to manipulate the activity of floccular MLIs during the performance of the VOR. Optogenetic suppression of MLI activity during a normally non-adapting visual-vestibular stimulus enhanced climbing fiber-evoked Ca^{2+} signaling in Purkinje cells. This enhanced response resembled that induced when mice experienced opposite-direction retinal slip (an adaptation-inducing stimulus). Consistent with the idea that enhanced Ca^{2+} signaling triggers behavioral modification, we observed that MLI activity suppression during this normally non-adapting stimulus led to an increase in VOR gain. This result suggests that inhibition from MLIs prevents climbing fibers from inducing Ca^{2+}-dependent plasticity when adaptation is unnecessary or inappropriate. It also implies that inhibition from MLIs must be relieved for climbing fibers to be effective in instructing learning. In support of this hypothesis, we found that ablating inhibition between MLIs prevented mice from adapting their VOR during opposite-direction visual-vestibular pairing. Therefore, we conclude that disinhibition of climbing fiber-evoked Ca^{2+} signaling in Purkinje cell dendrites is accomplished by an MLI-MLI microcircuit. These findings point to a decisive role for MLIs in regulating learning in the cerebellum.

Disclosures: K. Zhang: None. **A. Bonnan:** None. **G.G. Gross:** None. **D.B. Arnold:** None. **J.M. Christie:** None.

Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.11/N8

Topic: E.02. Cerebellum

Title: Changes in behavioral state account for the modulation of cerebellar learning by cannabinoid receptors

Authors: *C. ALBERGARIA[1], N. SILVA[3], D. M. DARMOHRAY[1], M. R. CAREY[2]; [2]Neurosci., [1]Champalimaud Ctr. For the Unknown, Lisbon, Portugal; [3]Champalimaud Ctr. for The Unknown, Lisbon, Portugal

Abstract: Attempts to identify cellular mechanisms underlying learning often include knocking out genes involved in candidate forms of synaptic plasticity and assessing subsequent effects on behavior. Within the cerebellum, multiple plasticity mechanisms have been proposed as cellular substrates of learning. For example, type 1-cannabinoid receptors (CB1Rs) mediate several forms of synaptic plasticity in the cerebellar cortex and have been implicated in cerebellum-dependent delay eyeblink conditioning in knockout experiments. However, recent work has shown that eyeblink conditioning is modulated by behavioral state, and global CB1KO mice are known to be hypoactive. We therefore asked to what extent altered locomotor activity vs. impaired CB1-dependent plasticity within the cerebellar cortex contribute to learning impairments in these mice. We find that eyeblink conditioning deficits in global CB1KOs can be fully accounted for by their hypoactivity. Impairments disappear when the level of locomotor activity is taken into account, and externally controlling running speed rescues learning. Moreover, both global and cerebellar granule cell-specific CB1KOs exhibit normal cerebellum-dependent locomotor adaptation. Our results suggest that the apparent effects of CB1R deletion on cerebellar learning are not due to direct effects on CB1-dependent plasticity, but rather, arise as a secondary consequence of hypoactivity. These findings highlight the importance of considering general changes in behavioral state as a powerful means through which individual genes contribute to complex behaviors, particularly in transgenic models.

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Poster

063. Cerebellum: Plasticity and Climbing Fibers

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 063.12/N9

Topic: E.02. Cerebellum

Title: Olivocerebellar connections in the Atlantic stingray (Dasyatis sabina)

Authors: *D. V. SCARTON[1], S. M. GRUBER[1], R. L. PUZDROWSKI[2];
[1]Col. of Sci. and Engin., Univ. of Houston-Clear Lake, Houston, TX; [2]Col. of Sci. and Engin., Univ. of Houston Clear Lake, Houston, TX

Abstract: The cerebellum of the Atlantic stingray (Dasyatis sabina) displays a unique complexity relative to other cartilaginous fishes (chondrichthyes). While the cerebellum of primitive chondrichthyes is a simple and smooth bi-lobed structure with a primary fissure dividing anterior and posterior lobes, advanced chondrichthyes like the stingray have a tri-lobed cerebellum in which the anterior lobe is further split into rostral and caudal lobules. Moreover, the cerebellar lobes in the advanced chondrichthyes are highly foliated. This specific morphological pattern may be due to functional specializations related to behavior in these animals. A prior study demonstrated that the stingray cerebellum receives neuronal inputs from many of the same sources as other chondrichthyes with a bi-lobed cerebellum, particularly from the diencephalic and brainstem nuclei, including the inferior olive. These inputs were also observed, however, to have a greater segregation in the complex cerebellum. While the accessory optic nuclei and spinal cord were shown to transmit to the anterior rostral and posterior lobules, respectively, the trigeminal and octavolateral nuclei were seen to send signals to the caudal lobule. Furthermore, previously undescribed midbrain neural centers provide massive inputs to the rostral and posterior lobes of the stingray cerebellum. These neural centers have not been described in other chondrichthyes, and are therefore suspected to have played a significant role in the evolution of the cerebellar complexity in the stingray. To date, there has been little research into the neuroanatomical connections of the cerebellum of advanced chondrichthyes. This study seeks to expand an understanding of the cerebellar structure, implicated functioning, and overall evolution to the chondrichthyes possessing a complex cerebellar structure, with a focus on the inferior olive. We are investigating these aims by retrospectively analyzing histological sections and performing additional neurotracing experiments to better characterize the inferior olive and its relationship to the cerebellum. Thus far, we estimate the inferior olive to extend approximately 2 mm from the entrance level of the IXth cranial nerve to the first spinal motor root in a stingray with a 12-in. disc width. The ventrolateral part extends this entire length, with the dorsolateral group of cells extending from the entrance level of the Xth cranial nerve to approximately the level of the obex (~1 mm). The inferior olive neurons appear multi-polar with

triangular soma. Other cell bodies appeared as more fusiform, elongated, or spindle-like, which is consistent with previous observations.

Disclosures: D.V. Scarton: None. **S.M. Gruber:** None. **R.L. Puzdrowski:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.01/N10

Topic: E.04. Voluntary Movements

Support: NIH Grant EY-012135

Title: Signals corresponding to bimanual movements in the posterior parietal cortex are shared across the hemispheres

Authors: *E. F. MOOSHAGIAN, C. D. HOLMES, L. H. SNYDER;
Dept. of Neurosci., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Primates use their arms in complex ways that frequently require coordination between the two arms. Here we asked whether and how information about bimanual reaching plans is shared between the parietal reach region (PRR) in each hemisphere. We recorded spikes and local field potentials (LFP) from PRR in both hemispheres simultaneously while monkeys planned and then executed unimanual and bimanual reaches. To assess information sharing between the hemispheres, we estimated the interhemispheric LFP-LFP and spike-LFP coherence. We found that information about the movement plans of the two limbs was shared across the hemispheres in a frequency and task-specific manner. Beta band LFP-LFP coherence between left and right PRR increased when planning a bimanual reach to a single target (bimanual-together) and decreased when planning a bimanual reach to two different targets (bimanual-apart), compared to baseline. LFP-LFP coherence in other frequency bands was unchanged. Spike-LFP coherence for bimanual-together movements was significantly higher than for bimanual-apart movements over most of the beta range. Further analyses showed that peak spike-LFP coherence occurred during bimanual-together trials when LFP lagged spikes by ~10 ms relative to the spikes. During bimanual-apart trials, spike-LFP coherence reached a nadir when LFP lagged spikes by ~15 ms relative to the spikes. These results are consistent with spikes from one PRR driving LFP in the other PRR, with a conduction delay occurring as spikes travel from one hemisphere to the other. Finally, a simple model that assumed LFP power to be influenced by a weighted sum of spikes from the contralateral and ipsilateral PRR predicted the observed beta band LFP responses. We conclude that bimanual reach planning involves interhemispheric communication between the left and right posterior parietal cortex.

Disclosures: **E.F. Mooshagian:** None. **C.D. Holmes:** None. **L.H. Snyder:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.02/N11

Topic: E.04. Voluntary Movements

Support: NIH Grant EY012135
Washington University Cognitive Computational and Systems Neuroscience Fellowship

Title: Inter-hemispheric communication between parietal reach regions in bimanual coordination

Authors: *J. KANG, E. MOOSHAGIAN, L. H. SNYDER;
Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Coordinating movements of the two arms is essential to our everyday activities. However, the neural circuitry for planning bimanual movements is not fully understood. We hypothesize that inter-hemispheric communication between the parietal reach regions (PRR) in each hemisphere via the corpus callosum facilitates bimanual coordination. To test this hypothesis, we compared behavioral performance and neural activity before and during reversible blockade of the callosal pathway connecting PRR in each hemisphere. We recorded spikes and local field potential (LFP) in PRR in both hemispheres simultaneously while an animal (rhesus macaque) made unimanual movements to a single target or bimanual movements to either a single target or two different targets. We identified callosal transmission from PRR by manganese tract tracing, and temporarily blocked callosal transmission between left and right PRR with focal injections of lidocaine. At the behavioral level, we observed overall faster reaction times in both unimanual and bimanual movements and a larger difference in reaction times of the two arms in bimanual movements during blockade. These results are consistent with that callosal connections between PRR facilitate bimanual movements via a competitive process such that the faster arm slows down to match the slower arm. At the neural level, we measured beta band LFP-LFP coherence of PRR in each hemisphere to get an estimate of inter-hemispheric communication between PRR. Prior to blockade, we observed three different levels of LFP-LFP coherence. The coherence was high when an animal made bimanual movements to a single target; intermediate when an animal made unimanual movements; and low when an animal made bimanual movements to two different targets. During blockade, the difference in LFP-LFP coherence in the two bimanual movements and unimanual movements was abolished. Our inter-hemispheric LFP-LFP coherence analysis suggests that bimanual coordination involves inter-hemispheric communication between PRR via the corpus callosum so that information about movement of each arm is shared in both hemispheres.

Disclosures: **J. Kang:** None. **E. Mooshagian:** None. **L.H. Snyder:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.03/N12

Topic: E.04. Voluntary Movements

Title: Influence of shared control parameters on interference during asymmetric dynamic perturbation

Authors: *P. C. DESROCHERS, A. T. BRUNFELDT, F. A. KAGERER;
Dept. of Kinesiology, Michigan State Univ., East Lansing, MI

Abstract: During complex bimanual movements, the action of one hand can influence the action of the other hand in a process called interference. Interference can be induced during bimanual movements by using a visuomotor perturbation in the right hand and observing directional error in the non-visible left hand. Interestingly, when participants experience a dynamic perturbation in the right hand, interference is minimal. It is possible that interference to visuomotor perturbations occurs in a reference frame that is shared between effectors, while interference to dynamic perturbations occurs in a reference frame specific to the limb or joint being perturbed. Our objective was to investigate whether conceptually sharing control parameters of a movement between limbs, and thus the reference frame in which the movement is planned, could elicit more interference between the hands when one limb experiences a dynamic perturbation. Participants were randomly assigned to either an experimental group, controlling independent cursors with each hand, using a KINARM bimanual robot, or a control group, controlling a single, central cursor, whose *y* position was defined as the average between both hands, while the *x* position was controlled only by the right hand. Vision of the hands was occluded. Trials consisted of simultaneous reaches from a home position to target positions directly forward or backward of the home position. Participants performed unperturbed reaches during two baseline blocks of 30 trials. Cursors representing hand position were first displayed for both hands and then removed for the left hand for the dual-cursor group, leaving only the right-hand cursor visible. The shared-cursor group first saw both hands and the central, shared cursor. Hand feedback was then removed in the second baseline block. In the exposure block of 250 trials, the right hand was perturbed using a dynamic perturbation, in which participants encountered a 25 N force in the *x* direction per 1 m/s reaching velocity in the *y* direction. Interference in the left hand was assessed at the moment of peak velocity, at the end of the initial ballistic movement, and at the end of the reach. Preliminary results show that at early points in the reaching movement, the dual-cursor group demonstrate greater directional error in the left hand. Conversely, the shared-cursor group demonstrate greater reaching error at the end of the reach. This may suggest that conceptually

sharing movement control parameters results in greater interference during feedback integration at movement phases that occur later in the reach.

Disclosures: P.C. Desrochers: None. **A.T. Brunfeldt:** None. **F.A. Kagerer:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.04/N13

Topic: E.04. Voluntary Movements

Support: Michigan State University Distinguished Fellowship

Title: Structural learning generalizes to a contralateral effector

Authors: *A. T. BRUNFELDT, P. C. DESROCHERS, F. A. KAGERER;
Michigan State Univ., East Lansing, MI

Abstract: Motor adaptation tasks have typically been studied in contexts where participants learn to adjust movement in response to a fixed perturbation in the relationship between motor outputs and sensory inputs. Such 'parametric' adaptation tasks require participants to learn a single solution that sets all movement parameters to fixed values optimized to the task goals. Interestingly, it has been shown that learning random perturbations belonging to the same structure (e.g. random rotations in visual feedback) facilitates adaptation to a subsequent novel, fixed perturbation of the same structure, and new tasks belonging to that structure will benefit from previous exposure to that structure. Since it is currently not known whether and to what degree learning a structure generalizes to the contralateral effector, we exposed participants to random visuomotor rotations in one hand, and then exposed the contralateral limb to a fixed perturbation of the same structure. We hypothesized that if the previously learned structure generalized to the contralateral effector, adaptation would progress faster in that hand. Right-handed participants (n=16) performed a center-out reaching task using a KINARM endpoint robot to two peripheral targets either 90^oor 270^o(10 cm reach). Participants controlled a cursor with their right hand projected on a screen that occluded vision of the hand. Following a veridical feedback baseline, participants experienced an exposure phase where one group (n=8) experienced a new random rotation in visual feedback in the interval {[-90, -70] U [-50, 50] U [70, 90]} every 4 trials for 240 trials while the other group received veridical feedback. All participants then performed the task in the left hand with exposure to a fixed 60^orotation for 40 trials. Adaptation was measured using initial directional error (IDE), normalized root-mean squared error (RMSE), movement time (MT), movement length (ML), and normalized jerk (nJ). IDE and RMSE were similar in the left hand for both groups early in exposure, but the structural training group moved faster, shorter, and smoother (reduced nJ). This indicates that structural

training does generalize to a contralateral effector, and adaptation is facilitated primarily through feedback processes. These results deviate from previous work that showed both feedforward and feedback processes are facilitated by structural learning. It is yet unclear whether this disparate result is specifically due to a lack of transfer of the feedforward component or to methodological differences in the uncertainty experienced during training.

Disclosures: A.T. Brunfeldt: None. **P.C. Desrochers:** None. **F.A. Kagerer:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.05/N14

Topic: E.04. Voluntary Movements

Support: Birmingham International Engagement Fund
US Department of Veterans Affairs
National Institutes of Health/National Institute of Neurological Disorders and Stroke

Title: Crossed corticospinal facilitation between arm and trunk muscles during bilateral tasks

Authors: *S.-Y. CHIOU[1], M. A. PEREZ[2,3,4];
[1]Sch. of Sport, Exercise, and Rehabil. Sci., Univ. of Birmingham, Birmingham, United Kingdom; [2]Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL; [3]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL; [4]Shirley Ryan Ability Lab., Chicago, IL

Abstract: We recently showed task-dependent interactions between corticospinal projections targeting unilateral arm and contralateral trunk muscles in intact humans. Here, we examined whether interactions between corticospinal projections targeting arm and trunk muscles change during bilateral contractions. Using noninvasive cortical and cervicomedullary stimulation, we examined motor evoked potentials (MEPs) and the activity in intracortical circuits (short-interval intracortical inhibition, SICI) in the resting erector spinae (ES) muscle when the contralateral arm performed 20 % of isometric maximal voluntary contraction (MVC) with biceps (BB) or triceps brachii (TB) - referred to as a unilateral task. During bilateral tasks, the ipsilateral arm performed 20 % of MVC with BB or TB, while the contralateral arm performed 20 % of MVC with BB or TB. We found that the size of cortically evoked MEPs in the ES increased during ipsilateral BB and contralateral TB contraction but decreased during ipsilateral TB and contralateral BB contraction. SICI decreased during ipsilateral BB and contralateral TB contraction and increased during ipsilateral TB and contralateral BB contraction. In contrast, cervicomedullary MEPs in the ES remained similar across conditions. Our findings reveal a

cortical origin for bilateral interactions between arm and trunk muscles, with pronounced effects when agonist and antagonist muscles are active. We hypothesized that these task-dependent reciprocal interactions reflect coupling between bilateral arm and trunk muscles during functionally relevant motor behaviors.

Disclosures: S. Chiou: None. **M.A. Perez:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.06/N15

Topic: E.04. Voluntary Movements

Support: European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement N° 642961.
Innovative Training Network 'Perception and Action in Complex Environment' (PACE)
FRS-FNRS PDR grant

Title: Hand coordination in space overrules the optimization of variability and effort during bimanual tracking

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Abstract: Optimal control theory suggests that minimizing motor costs such as the variability of movement and effort is an objective for every movement. However, it is well established that during bimanual tasks, humans have a natural tendency to generate similar hand movements. Here we examined to which extent this natural tendency impacts on the minimization of effort and variability. Participants were requested to track a moving target by means of a single cursor controlled simultaneously by the two hands. Two types of hand-cursor mappings were tested: one in which the cursor position resulted from the average location of two hands (MEAN), and one in which horizontal and vertical positions of the cursor were driven separately by each hand (SPLIT). First, our results are consistent with that of previous studies on manual dexterity, with dominant hand being better in unimanual-tracking than the more variable non-dominant hand. More interestingly, instead of exploiting this effect by increasing the use of the dominant hand

during bimanual cooperative tasks, the contributions from both hands remained symmetrical. Indeed, for both mappings, and even after six minutes of practice, right and left hands remained strongly correlated and performed very similar movements in extrinsic space. Persistence of this bimanual coupling demonstrates that participants preferred to maintain similar movements at the expense of unnecessary movements (SPLIT) or increase in noise from the non-dominant hand (MEAN). Altogether, during bimanual tracking, coordination of both hands in extrinsic space is therefore more important than minimizing motor costs associated with variability and effort.

Disclosures: **J. Mathew:** None. **F. Danion:** None. **A. De Rugy:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.07/N16

Topic: E.04. Voluntary Movements

Support: Marie Skłodowska-Curie
Torsten Söderbergs Stiftelse

Title: Rapid temporal retuning of internal models after exposure to sensory delays

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Abstract: Prevalent theories of motor control posit that our brain uses internal models to predict the sensory consequences of our self-generated movements (Bays and Wolpert, 2007; Franklin and Wolpert, 2011). Due to these predictions, the perception of the sensory feedback of our movements feels less intense and ticklish compared to identical stimuli of external origin (Blakemore et al., 1999; Bays and Wolpert, 2008; Shergill et al., 2003). It has previously been shown that these predictions are time-locked to our movements and even small delays between the movement and the sensory feedback can significantly reduce the attenuation and increase the ticklishness of the latter (Blakemore et al., 1999; Bays et al., 2005). We tested whether the brain can learn to predict a new temporal relationship between the movement and its tactile feedback. Sixty volunteers participated in two experiments. The first experiment included a force discrimination task, in which the participants were asked to press a sensor with their right index finger that delivered a tap (2 N) on their left index finger. The tap was applied either immediately (0 ms) or with a 100 ms delay. Following, a second tap (between 1 and 3 N) was applied on the left index finger and participants had to indicate which tap felt stronger using a foot pedal. Prior to the force discrimination task, participants were exposed to either a 0 ms or a 100 ms delay between the press of the right index finger and the tap on the left index finger in a training

session of 500 exposure trials. In the second experiment, blindfolded participants were asked to move the arm of a robot with their right hand and received tactile stimulation on their left forearm via the arm of a second robot. The second robot copied the movement of the first robot either without delay (0 ms) or with a 150 ms delay. In a two-alternative forced choice task, participants produced two successive stimuli on their left forearm (one with 0 ms and one with 150 ms delay) and they indicated which stimulus felt more ticklish.
In Experiment 1, we found a decreased attenuation of immediate touch (N=30, t(29)=3.03, p=0.005), and increased attenuation of delayed touch (N=30, t(29)=-3.5156, p=0.002) after exposure to the delay. We further observed that the reduction in the attenuation of immediate touch was correlated with the increase in the attenuation of delayed touch **(***r*=0.473, *t*(28)=2.84, *p*=0.008). In Experiment 2, we found an increase in the ticklishness of the immediate touch after exposure to the delay (N=30, t(29) =2.28, p= 0.030).
Our findings suggest that the brain can rapidly update the internal models by retuning the temporal relationship between our movements and their sensory consequences.

Disclosures: K. Kilteni: None. **C. Houborg:** None. **H. Ehrsson:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.08/N17

Topic: E.04. Voluntary Movements

Title: Tonic electromyography in task irrelevant muscles differs between successful and failed stopping

Authors: *M. FISHER, I. UTRECHT, I. GREENHOUSE;
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Abstract: The ability to stop an initiated movement is essential to daily life. Stopping can be measured in the laboratory with the Stop Signal task. Transcranial magnetic stimulation (TMS) studies have shown stopping speech, saccades, or manual responses are associated with suppressed excitability in task-irrelevant muscles. This widespread suppression of the motor system is attributed to the prefrontal-subthalamic 'hyperdirect' pathway, which has a potent influence over basal ganglia inhibitory output to the motor system. However, TMS may not be the most effective means to map the temporal profile of motor suppression during stopping. In this experiment, we measured electromyography (EMG) in a tonically contracted task-irrelevant muscle with the aim of detecting a marker of motor suppression during stopping. Participants (n = 16; 10 female, age 23.8 ± 2.5 yrs) performed a simple Go task (30 trials) and then a simple Stop Signal task (108 trials) with each hand (hand order counterbalanced). EMG was collected from both first dorsal interossei. Participants were trained to maintain tonic EMG at

approximately 10% of their maximal voluntary contraction by squeezing a ball between the thumb and index finger of the non-responding hand. The tonic contraction was maintained for the duration of the tasks. On each trial of both tasks, participants responded to a Go stimulus (.5 s green box) by pressing a button using a lateral index finger movement. On 1/3 of Stop Signal task trials, a stop signal (red X) appeared over the Go stimulus at a dynamic delay that tracked stopping performance. Participants were instructed to respond quickly to the Go stimulus but to try to stop when stop signals appeared. A predicted decrease in tonic EMG following stop signal onset for successful stop trials was not observed. However, the mean rectified tonic EMG in the time window -100 to 500 ms relative to the stop signal was significantly greater on failed stop compared to successful stop trials ($F[1,15] = 9.2$, $p < 0.01$). This effect did not differ between hands, and there was no interaction. The observed difference in tonic EMG in a task-irrelevant muscle suggests stopping success is associated with overall motor system excitability.

Disclosures: **M. Fisher:** None. **I. Utrecht:** None. **I. Greenhouse:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

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Program #/Poster #: 064.09/N18

Topic: E.04. Voluntary Movements

Support: São Paulo Research Foundation–FAPESP fellowship 2018/26007-5
CNPq fellowship 311602/2018-5

Title: Interlimb facilitation of handgrip strength is scaled to opposite arm force generation

Authors: G. C. TOFFANO[1], T. P. DOS SANTOS[1], R. L. SAINBURG[2], ***J. E. DE ARAUJO**[1]; [1]Hlth. Sci., Univ. of São Paulo, Ribeirao Preto, Brazil; [2]Penn State Univ., University Pk, PA

Abstract: There has been general agreement that during unilateral voluntary contractions, unintended muscle activity can be observed in the contralateral homologous muscle (Shinohara et al. 2003, for review). This phenomenon is named motor overflow, that is the natural tendency for a resting limb to move during movement of the opposite limb. It is thought to be influenced by inter-hemispheric interactions and intracortical networks within the 'resting' hemisphere (Perez and Cohen 2008). Perez and co-workers (Long J. et al., 2016) had shown that when healthy young participants performed an isometric contraction at greater than or equal to 70% of maximum voluntary isometric contraction (MVIC) of a homologous muscle, there is an Interhemispheric Facilitation (IHF) showed through TMS. We now hypothesize that such interhemispheric facilitation might occur not only in homologous muscle and in more general patterns of tasks. We predicted that isometric resistance to finger flexion in one hand should facilitate (increase) the grip strength at the other hand performs after repetitive handgrip tasks in

a dynamometer. To test this prediction, participants performed four hand grip muscle contractions, each one sustained by five seconds with five seconds to rest, in a hand-held dynamometer. Before the fourth contraction, the participants produced an isometric force with the opposite hand index finger (percentage of MVIC determinate by the grip strength obtained in the first handgrip contraction). Ninety right-handed participants were divided into four groups (age 17-22, mean 20.35± 1.73 SE). Two resistance groups performed the grip strength task with the right hand (isometric finger flexion at the left) or vice versa. Two controls groups did the same, but with no isometric finger flexion in the opposite limb. In the four groups, the grip strength decreases in the second and third contraction, but in the resistance group, after the participates did the finger flexion at 70% or higher of MVIC, the grip strength increases in the fourth contraction. The grip strength increase is scaled with opposite hand isometric force, and we do not see the increase when the force is less than 70% of MVIC or in the control groups. In our participants, the effect is more robust for the right-hand grip of men and in the left-hand grip of women. These results might be explained by IHF, and as we do not use only homologous muscles, we suggest that this might result from a dopamine-mediated increase in strength "vigor" (Panigrahi B et al., 2015).

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Poster

064. Motor Systems: Fine Manual Control

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Program #/Poster #: 064.10/N19

Topic: E.04. Voluntary Movements

Title: Bimanual coupling between motor execution and kinesthetic illusion of movement

Authors: *M. BOVE[1], F. GARBARINI[2], M. BIGGIO[1], A. BISIO[1];
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Abstract: Muscle tendon vibration at 80Hz, activating muscle spindles, is able to evoke a kinesthetic illusion of movement whose complexity strictly depends by the number of vibration sites active at a level of a specific body segment. When people simultaneously draw lines with one hand and circles with the other hand, both trajectories tend to assume an oval shape, showing that hand motor programs interact (the so-called "bimanual coupling effect"). The goal of the present study was to investigate how motor parameters (drawing trajectories) change during the bimanual coupling between the real motor execution and the kinesthetic illusion of movement. To this aim, we designed a multisite proprioceptive stimulation system able to co-vibrate four different muscle tendons at the level of the wrist. We recruited eight young right-handed subjects. They were asked to place their right hand in a position habitually used when writing

with a pencil. Then, the forearm was immobilized in this position. Assuming that movement trajectories originates from the sum of the vectors of each stimulated muscle tendon, we designed patterns of stimulation evoking illusion of lines and circles. In the real modality, subjects performed right hand movements (lines or circles) and, simultaneously, congruent or non-congruent left hand movements, respectively. In the kinesthetic illusion modality, subjects performed only left hand movements and, simultaneously, received a congruent or non-congruent illusion of right hand movements. Behavioral results showed a similar interference of both the real and the illusory movements on the actually executed circles and lines. These findings indicate that bimanual coupling effect is also present in the kinesthetic illusion condition and suggest that kinesthetic illusion of movement shapes the motor coordination between the two hemispheres.

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Poster

064. Motor Systems: Fine Manual Control

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Topic: E.04. Voluntary Movements

Support: GINOP-2.3.2-15-2016-00022
EFOP-3.6.1-16-2016-00004

Title: Body position does not affect jerk decomposition in upper limb cycling

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Abstract: In multi-joint limb movements the decomposition of the endpoint jerk into components related to successive derivatives of joint angular velocities and limb configuration may give different results depending on external conditions as movement directions, body position or motor impairment. Such decomposition has been earlier presented for reaching arm movements when the integral of the squared total endpoint jerk was decomposed into 4 components. It was found that 1 of the components dominates the total jerk and that the relative contribution of 4 components is a predictor of motor impairments after stroke (IEEE Trans. on Neural Syst and Rehab. Eng, 2017; V. 25, pp. 798-810).
Here we present the effect of body position on the relative contribution of the jerk components when participants performed arm cranking movements. Arm cranking is a bi-manual multi-joint motor task, which is applied in medical rehabilitation and in sport training. We investigate the

smoothness and the structure of smoothness of this type of movements applying an unusual device in which the two handles (left and right) are unconnected and thus the 2 arms could crank independently. Twelve able bodied persons (6 male, 6 female, 26.9 +/-6.4 years) performed arm cranking on this custom made device. The device made it possible to perform the movement in sitting and supine body positions. The participants cycled bimanually in the 2 body-positions. In both positions they cranked for 30sec. Cycling cadence was 60rpm. The order of positions in which cycling was performed were random. Coordinates of markers placed on the participant's arm were recorded with 100Hz, using a movement analyzer system (ZEBRIS, Germany) and joint angles were computed from marker coordinates. The relation between endpoint (hand) velocity and joint angular velocities, was given by the Jacobian. Successive derivatives of this relation provided the components of the endpoint jerk. 2 components dominated the endpoint jerk, in contrast to 1, which was found in reaching movements. We calculated a multiple ways mixed factor analysis of variance (ANOVA) to compare jerk decomposition for cranking in sitting and supine positions. It was found that body position does not have an effect on jerk decomposition. The relative contribution of the jerk components to the endpoint jerk did not differ significantly when cranking was performed in the 2 different body positions. This holds for both arms. Arm cranking in supine position may be performed by people who are unable to perform it sitting, but even so, the movement is equally well controlled in terms of the combination of jerk components related to joint angular velocities, accelerations and jerks.

Disclosures: L. Botzheim: None. **D. Piovesan:** None. **J. Laczko:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.12/N21

Topic: E.04. Voluntary Movements

Title: Influence of upper limb contractions on corticospinal excitability of trunk muscles

Authors: P. SHARMA, ***C. L. HAGGETT**, P. H. STRUTTON;
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Abstract: Contractions of upper limb muscles of one arm increase the excitability of corticospinal projections to the contralateral erector spinae (ES) muscles; this is known as crossed-facilitation. However, many functional interactions between the limbs and the trunk involve bilateral contractions. The extent to which bilateral contractions of upper limb muscles influence the degree of crossed-facilitation of trunk muscles remains unexplored. We investigated the effects of upper limb contractions on corticospinal excitability of ES muscles in 14 active healthy volunteers. Transcranial magnetic stimulation was applied over the ES representation of the primary motor cortex at 120% motor threshold and electromyographic

(EMG) activity was recorded from ES and rectus abdominis (RA) contralateral to the dominant hand. Participants performed low level isometric contractions in six tasks: unilateral elbow flexion/extension of the dominant arm, bilateral elbow flexion/extension, dominant arm flexion with non-dominant arm extension and non-dominant arm flexion with dominant arm extension. MEP amplitudes for each bilateral contraction were normalised to the respective unilateral task. Bilateral flexion was associated with an increase in ES MEPs (mean±SEM 165.22±34.32%), this was greater than the size of MEPs obtained during the dominant flexion/non-dominant extension task (112.37±13.89%). Furthermore, despite not targeting the hotspot for RA, MEPs were elicited during all tasks. The pattern of increase in MEPs in RA was similar during elbow extension tasks. Bilateral extension was associated with an increase in RA MEPs (465.04±163.21%) and was greater than the size of MEPs obtained during the dominant extension/non-dominant flexion task (246.01±99.84%).
We further assessed if the crossed-facilitation effect was related to the degree of physical activity, since it has been shown that unilateral muscle strength training increases contralateral voluntary strength. Physical activity was determined using the International Physical Activity Questionnaire (IPAQ); no significant relationship was found between IPAQ scores and crossed-facilitation effect in any of the tasks. Further, IPAQ scores did not correlate with maximum voluntary contractions in all assessed muscle groups, suggesting that a more specific questionnaire is required to differentiate types of training undertaken. It remains to be established whether specific types of physical activity have effects on limb-trunk crossed-facilitation. By exploring this further, it may be possible to develop targeted training for those with impaired trunk control, but good upper limb control.

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Poster

064. Motor Systems: Fine Manual Control

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Topic: E.04. Voluntary Movements

Support: National Institutes of Health/National Institute of Neurological Disorders and Stroke
US Department of Veterans Affairs
Craig H. Neilsen Foundation

Title: Robotic characterization of movement asymmetry in humans with spinal cord injury

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[2]Shirley Ryan Ability Lab., Chicago, IL; [3]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Humans with cervical spinal cord injury (SCI) show asymmetrical motor impairments in arm function. However, current assessments of movement asymmetry largely rely on a clinician's subjective interpretation and the patient's self-report that both have poor reliability and lack sensitivity to discriminate subtle deficits. Here, we used a robotic exoskeleton and a virtual reality system to assess movement asymmetry during self-paced reaching movements to multiple targets in 24 individuals with chronic incomplete cervical SCI and 22 uninjured age-matched control subjects. We found that SCI subjects showed prolonged movement duration during arm acceleration and deceleration compared with controls in both arms. Specifically, SCI subjects exhibited an additional increase in movement duration during arm deceleration when reaching to the target requiring elbow extension but not elbow flexion. The endpoint accuracy was decreased and trial-to-trial variability was increased when reaching to the target requiring elbow extension. The more affected arm showed prolonged arm deceleration compared with the less affected arm of SCI subjects during elbow extension. Our results suggest that deficits in movement kinematics are more pronounced during the arm deceleration phase of reaching movements in the more affected arm of SCI participants, consistent with the view that demands for precise control during reaching gradually increases when approaching the target. We propose that the magnitude of extensor muscle weakness might be a good predictor for detecting asymmetrical impairments in upper-limb function following SCI.

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Poster

064. Motor Systems: Fine Manual Control

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Topic: E.04. Voluntary Movements

Support: European Social Fond for Germany and Sächsische Aufbaubank-Förderbank of the Free State of Saxony (Project-Number: 100310502)

Title: Complexity matching in asymmetric bimanual movement tasks is reduced in individuals with mild cognitive impairments

Authors: *J. RUDISCH, K. MÜLLER, C. VOELCKER-REHAGE;
Chemnitz Univ. of Technol., Chemnitz, Germany

Abstract: Introduction: Intact sensorimotor systems have large numbers of neuromuscular degrees of freedom they can exploit for a motor task. Consequently, they show a complex

variability structure (i.e., random fluctuations) of force output when producing a constant force. Non-constant force production (e.g. sine-waves) in contrast requires specific muscle activation patterns and therefore reduction of behavioral complexity. Complexity has been shown to be reduced for constant and increased for non-constant force production tasks in aged as compared to young individuals.
In bimanual tasks, when producing a sine-wave force simultaneously to a constant force (asymmetric task), interhemispheric cross-talk may result in an involuntary reduction of the degrees of freedom of the constant task to match the complexity of the sine-wave task dynamics. Declines in interhemispheric connectivity, as have been shown for individuals with mild cognitive impairments (MCI), may reduce this interhemispheric complexity matching. We therefore investigated whether presence of MCI affects complexity matching in asymmetric bimanual force production tasks.
Methods: We investigated 20 younger controls (YA, 21-30 years), 20 cognitively healthy older adults (OA, 80-87 years, Montreal Cognitive Assessment [MoCA] 27-30), and 20 OA with MCI (OA_{MCI}, 80-85 years, MoCA 20-24) performing a symmetric (ST) and an asymmetric (AT) bimanual force control task. In ST, both hands performed a constant force matching task (12% of MVC). In AT, one hand performed a constant and the other a sine-wave task (5-12% of MVC). Detrended fluctuation analysis (DFA) was applied to assess long-range dependencies of signals, i.e., whether the force output at a certain point in time is dependent on previous values or random.
Results: YA and OA, but not OA_{MCI}, showed significant decrease in complexity structure during constant force production in the asymmetric as compared to the symmetric task.
Discussion: In the asymmetric task, decrease of constant force complexity may be due to complexity matching of the sine-wave force due to interhemispheric cross-talk. Reduced complexity matching in OA_{MCI} may be due to disease-related reduced interhemispheric connectivity. Further studies should measure neurophysiological correlates to investigate a direct relationship between interhemispheric connectivity and bimanual complexity matching more in detail.

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Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.15/N24

Topic: E.04. Voluntary Movements

Title: Getting behind the mirror during mirror therapy: What happens to the unseen hand?

Authors: *J. M. KIM, S.-H. YEO, T. D. PUNT;
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Abstract: Following hemiparetic stroke, performing bimanual movements while receiving illusory feedback about the affected limb via a mirror (mirror visual feedback or MVF) is known to have therapeutic benefits (Thieme et al., 2019). *Visual capture* of the unimpaired limb in the apparent position of the impaired limb creates the illusion that one is actually viewing the impaired limb; the performance of bimanual movements appears to further strengthen this illusion by providing congruence between one's motor intentions and the resulting visual feedback. However, this approach can create a conflict between vision and proprioception (Holmes et al., 2004) and few studies to date have examined the impact of MVF on motor control of the limbs. We conducted two experiments examining the kinematics of bimanual circle-drawing movements in unimpaired participants with and without mirror visual feedback. In both experiments, the *mirror* condition led to substantial positional drift of the *unseen* hand; this was not the case when an opaque screen replaced the mirror (i.e. the *no mirror* condition). The positional drift was greatest when movements were made in the presence of a visual template (Experiment 1) than when this was removed (Experiment 2). Furthermore, the small but reliable asynchronies known to be modulated by vision during bimanual circle-drawing movements (Swinnen et al., 1996) were replicated in both experiments but were more pronounced in the *mirror* than the *no mirror* condition. This research demonstrates the modulatory effects that MVF can have on motor control of the unseen hand in unimpaired individuals. MVF leads to a rapidly-induced and powerful illusion that appears to affect both central and peripheral aspects of motor control. Our findings provide the basis for similar studies in impaired participants (e.g. hemiparesis following stroke).
Holmes NP, Crozier G, Spence C (2004) When mirrors lie: "visual capture" of arm position impairs reaching performance. Cognitive, Affective and Behavioral Neuroscience 4:193-200.
Swinnen SP, Jardin K, Meulenbroek R (1996) Between-limb asynchronies during bimanual coordination: effects of manual dominance and attentional cueing. Neuropsychologia 34:1203-1213.
Thieme H, Morkisch N, Mehrholz J, Pohl M, Behrens J, Borgetto B, Dohle C (2019) Mirror Therapy for Improving Motor Function After Stroke. Stroke 50:e26-e27.

Disclosures: J.M. Kim: None. **S. Yeo:** None. **T.D. Punt:** None.

Poster

064. Motor Systems: Fine Manual Control

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Program #/Poster #: 064.16/N25

Topic: E.04. Voluntary Movements

Title: The control of bimanual movements using covert visual attention

Authors: S. D. SARDAR, S.-H. YEO, S. C. NESBITT, ***T. D. PUNT**;
Univ. of Birmingham, Birmingham, United Kingdom

Abstract: The visual control of bimanual movements creates a clear challenge for the visuo-motor system; while bimanual movements tend to be organised as a single unit (i.e. they are *coupled*), *overt* vision can only be directed towards one target at a time. Previous research suggests that individuals make eye movements as limb movements unfold in order to optimise control, but *covert visual attention* is also thought to play a substantial role. In order to investigate this contribution further, we examined the control of unimanual and bimanual movements in the absence of eye movements. Unimpaired right-handed participants made unimanual or bimanual reach-to-point movements to touchscreen targets presented on the left and/or right of a fixation cross; electro-oculography monitored fixation and any errant saccades were recorded. The kinematics of limb movements were recorded using a motion capture system. Errant saccades were observed on 22% of trials, were more likely to occur for bimanual movements (65%) and were far more likely to be directed *rightwards* (74%). Trials including these errant saccades were subsequently excluded from further analysis. Kinematic limb data revealed bimanual movements to be coupled and scaled according to target characteristics; for example, movements to small targets were slower than those to large targets. Interestingly, non-dominant (left) arm movements were more accurate than dominant (right) arm movements. Furthermore, bimanual movements were more accurate than unimanual movements for both limbs. We suggest the rightward bias in errant saccades together with the reduced accuracy for the dominant limb reflects the relative dependency of this limb on *overt* visual guidance. On the other hand, superior accuracy of the non-dominant limb under the same conditions perhaps reflects the known proprioceptive advantage for that limb. We speculate that the superior accuracy observed overall for bimanual (compared with unimanual) movements is explained by a shift in the weighting of sensory processing from (less precise) vision to (more precise) proprioception with increasing task demands.

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Poster

064. Motor Systems: Fine Manual Control

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Program #/Poster #: 064.17/N26

Topic: E.04. Voluntary Movements

Title: Age-dependent changes in bilateral coordination: A kinematic and electroencephalography study

Authors: P.-C. SHIH[1], V. NIKULIN[1], C. J. STEELE[2], C. GUNDLACH[3], J. KRUSE[4], A. VILLRINGER[5], ***B. SEHM**[1];
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Abstract: In-phase (IP) and anti-phase movements (AP) represent fundamental bilateral coordination modes: bilateral homologous muscles contract synchronously during IP, and alternately during AP. Differential neural control processes have been suggested to govern both modes, with a common neural generator for both limbs during IP and a more independent control during AP. Here, we investigated whether aging differentially affects these movement patterns using simultaneous kinematic measurement and EEG.
23 young and 23 elderly right-handed participants performed a metronome-cued bilateral circle-drawing task with IP (mirror-symmetrical direction) and AP (non-mirror direction) movements using an exoskeleton device (KINARM; BKIN Technologies Ltd, Canada). Each movement condition was presented for 15 seconds and repeated twenty times in a randomized order. An inter-limb synchronization index was calculated to quantify bilateral coordination. Neuronal activity was assessed by task-related power changes in alpha (8-12Hz), lower-beta (13-20Hz), and higher-beta (21-30Hz) bands. Directional connectivity between bilateral motor cortices was estimated with the phase slope index (PSI) between C3 and C4. The relationship between PSI and inter-limb synchronization was examined using a linear mixed model with PSI, group, and condition as fixed factors, and a random subject intercept.
Inter-limb synchronization decreased during AP movements compared to IP in both young and elderly participants, but it reduced stronger in the elderly group (group x condition: $F(1,45) = 5.953$, $p = 0.018$). On a neural level, the same interaction was observed for the power in alpha and higher-beta bands, although in different directions: young adults showed decreased alpha power over the non-dominant motor area, whilst the elderly showed decreased higher-beta power over midline frontoparietal regions. Moreover, an inverted-U relationship was observed between PSI and inter-limb synchronization, suggesting that a stronger directional M1 connectivity predicts a lower inter-limb synchronization performance. This effect specifically is present in the AP condition and stronger in the elderly (group x condition x PSI^2: $t = -2.710$, $p < 0.001$).
In sum, bilateral AP movements are more sensitive to age-related motor decline, which is reflected by differential changes in alpha and higher-beta band power. Stronger interhemispheric directional connectivity is associated with the decrease in AP coordination performance in the elderly. Our findings provide insight into neural mechanisms of age-related decline in bilateral coordination and may foster novel rehabilitation strategies.

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Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.18/N27

Topic: E.04. Voluntary Movements

Title: Spatial constraints of a visuomotor task during bilateral transfer learning

Authors: *R. N. ADDISON[1], A. W. VAN GEMMERT[2];
[2]Kinesiology, [1]Louisiana State Univ., Baton Rouge, LA

Abstract: Introduction: Logic suggests that spatial location constraints affect motor skill learning. Studies have addressed spatial location constraints and others, bilateral transfer of learning (a learned skill with one limb transfers to the untrained contralateral limb; usually benefits of the transfer are unequal between limbs), but none have tried to address the effects of the spatial location constraints on bilateral transfer. **Purpose:** The purpose of this study was to explore whether the spatial location of the workspace affects bilateral transfer of a visuomotor task.**Methods:** Sixty-four right-handed young adults were recruited. They were assigned randomly to a group training the task in the workspace between the two shoulders or a group training the task in the workspace on the left or the right of workspace between the shoulders. Each group was subdivided into four equal groups of 8. Two of the subgroups trained the task with the right hand while the other two trained it with the left hand. Of these two subgroups, one group trained and performed the task during retention with the same hand (ipsilateral groups) whereas the other group trained and completed the task with different hands (bilateral groups). Performance (Movement time (MT), Normalized jerk (NJ), and Pathlength (PL)) before and after training was collected to determine learning effects. A 2(Workspace) x 4(RRR, LLL, LRL, RLR) x 2(pre-/post training test) ANOVA with group and workspace as between factors and test-time as within factors was performed.**Results:** Results indicated retention occurred for all groups on all variables (NJ ($F(1,56)=31.801$, $p<0.001$), MT ($F(1,56)=51.220$, $p<0.001$) and PL ($F(1,56)=27.904$, $p<0.001$)). Retention between groups differed for only PL ($F(3,56)=5.307$, $p=0.003$). Whereas retention between workspace differed for only NJ ($F(1,56)=4.149$, $p=0.046$). A two-way interaction between group and workspace was observed for PL ($F(3,56)=3.147$, $p=0.032$) and NJ ($F(3,56)=2.931$, $p=0.041$). **Discussion:** These results seem to indicate that workspace constraints play a role in motor skill learning, but more research, combining workspace constraints and transfer of learning conditions is needed to get a more comprehensive understanding.

Disclosures: R.N. Addison: None. **A.W. Van Gemmert:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.19/N28

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant to LES

Title: One hand or two: Using multivariate pattern analysis to locate brain regions that distinguish between unimanual and bimanual tasks

Authors: *D. J. GORBET[1], L. E. SERGIO[2];
[2]Sch. Kinesiol & Hlth. Sci., [1]York Univ., Toronto, ON, Canada

Abstract: Throughout the literature, there are many studies demonstrating that unimanual and bimanual movements activate similar regions of the brain. However, far less is known regarding how patterns of activity within these regions might differ for tasks using one versus both hands. In the study presented here, participants underwent fMRI while performing a button press task using either their dominant right hand (i.e. the unimanual task) or both of their hands (i.e. the bimanual task). One imaging run was used as a functional localizer to isolate regions of interest (ROIs) that were significantly active for both tasks with a conjunction analysis at the group level. This analysis revealed eleven regions that were active for both tasks. The remaining four imaging runs were input into a ROI multivariate pattern classification analysis to determine whether activity within any of the ROIs could distinguish between the two tasks. Regions in which patterns of activity could be used to decode the performed task included the right postcentral gyrus/inferior parietal lobule, and lobules VI and VIII of the left cerebellar hemisphere (permutation analysis, $p<0.05$). Significant classification accuracy was not obtained in the other ROIs tested which included left hemisphere primary sensorimotor cortex, medial premotor cortex, insula, putamen, and thalamus regions, or within the right cerebellar hemisphere. These results suggest that a number of brain regions that control unimanual and bimanual movements overlap. Only a subset of the network involved in manual control appears to provide information about whether one or two hands are performing the movements.

Disclosures: D.J. Gorbet: None. **L.E. Sergio:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.20/N29

Topic: E.04. Voluntary Movements

Title: Using machine learning to investigate sexual dimorphisms in fine motor control during rat string-pulling behavior

Authors: R. I. LAKE[1], N. S. ADAMCZYK[1], M. K. LORD[1], A. A. BLACKWELL[1], J. R. OSTERLUND[1], P. M. HASTINGS[2], I. Q. WHISHAW[3], *D. G. WALLACE[1];
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Abstract: Rats spontaneously engage in sequentially organized string-pulling behavior that depends on sensorimotor function and bimanual coordination. Sexual dimorphisms have been observed in other organized spontaneously occurring behaviors (e.g., food protection behavior) but have yet to be investigated in string-pulling behavior. Quantifying the organization of these behaviors depends on frame-by-frame manual digitization of body parts. This study examined the accuracy of DeepLabCut machine learning algorithm, which infers the vast majority of hand positions from a small number of annotated frames, in tracking female and male rat string-pulling behavior. Preliminary data analysis demonstrates that DeepLabCut accurately tracks hand position during string-pulling behavior. From this, sexual dimorphisms can be observed in the movement organization. These results will be discussed in the context of organizational and activational effects of sex hormones on spontaneously occurring behaviors. This work establishes a foundation for future research investigating sexual dimorphisms in neurological disorders that target motor systems and efficacy of therapeutic interventions.

Disclosures: R.I. Lake: None. **N.S. Adamczyk:** None. **M.K. Lord:** None. **A.A. Blackwell:** None. **J.R. Osterlund:** None. **P.M. Hastings:** None. **I.Q. Whishaw:** None. **D.G. Wallace:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.21/N30

Topic: E.04. Voluntary Movements

Title: Disruptions of fine motor control in string-pulling behavior following middle cerebral artery occlusion (MCAO) stroke in the rat

Authors: *A. A. BLACKWELL[1], M. L. HART[2], I. Q. WHISHAW[3], J. L. CHEATWOOD[2], D. G. WALLACE[1];
[1]Northern Illinois Univ., DeKalb, IL; [2]Southern Illinois Univ., Carbondale, IL; [3]Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Stroke is a leading cause of long-term disability in humans and unilateral stroke frequently results in bilateral impairment of fine motor control. Many behavioral tasks used for rodent models of stroke assess only a single limb; however, recent work has demonstrated that the bilateral hand-over-hand movements to reel in a string can access the fine motor control of both hands as well as their temporal integration. Rats spontaneously engage in string-pulling behavior featuring highly organized reach and withdraw phases of movement and with reinforcement the task is performed on demand. After focal sensorimotor cortical damage to the forelimb motor cortex, persistent deficits are observed in rat string-pulling behavior, including misses while pulling in the string and changes in movement topography (i.e., distance and concentration). The current study examines the impairments and recovery of string-pulling behavior after middle cerebral artery occlusion (MCAO) stroke, which largely spares the forelimb motor cortex but frequently involves the lateral striatum. Detailed movement analyses revealed that the bilateral organization of string-pulling is changed as is the dexterity of both hands. Rats missed the string more often with both hands, and when the string was missed on the impaired side, rats continued to cycle through the subcomponents (i.e., pulls and pushes) of string-pulling behavior as if the string was grasped in the hand. Rats failed to make a grasping motion with the impaired hand when the string was missed and instead, demonstrated an open raking-like motion. Further, a reduction in the transitions from one movement subcomponent to the next resulted in a reduction in the use of movements as a pulling cycle progressed in the hand contralateral to the stroke. No differences were found in motivation or time to complete the string-pulling task to obtain a reward, demonstrating the importance of using a detailed functional analysis of movement to detect changes in performance. String-pulling behavior is sensitive at detecting changes in fine motor control following MCAO. This research provides a foundation for future work investigating other models of stroke and evaluating the efficacy of therapeutic interventions that enhance neuroplasticity.

Disclosures: A.A. Blackwell: None. **M.L. Hart:** None. **I.Q. Whishaw:** None. **J.L. Cheatwood:** None. **D.G. Wallace:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.22/DP07/N31

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: E.04. Voluntary Movements

Support: NIH Grant NS061963

Title: Two thumbs up: How mice handle food with their first digits and forepaws

Authors: *J. M. BARRETT, M. G. RAINERI TAPIES, G. M. G. SHEPHERD;
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Abstract: The pad-like superficial appearance of the diminutive first digit (D1) of the Mus musculus manus belies a musculoskeletal anatomy sufficiently thumb-like to suggest ethological importance for handling small objects such as seeds. To explore this possibility, we analyzed movies of mice eating seeds. We identified multiple distinct movements and postures of the forelimbs and D1 associated with seed-handling. First, seed-handling involved rapid cycling between phases of higher and lower levels of oromanual activity. Second, to grip seeds, mice mainly used either a pincer-like grasp with D1 behind the seed, or a "thumb-holding" grasp with D1 apposed to the side of the seed. Third, while feeding, mice frequently executed rapid, stereotyped movements of the forepaws, including a "sniffing maneuver" to briefly bring the seed directly under the nares, and a "regrip maneuver" to deftly reposition the D1 and other digits on the morsel. These aspects of seed-handling behavior were similar for freely moving and head-fixed mice. Video analysis of a fox squirrel (Sciurus niger) handling nuts revealed movements with both similar and distinct features, including an extreme form of thumb-holding. The findings reveal an outsized role of D1 in mouse seed-handling movements, and show how these complex movements are composed of simpler stereotyped elements.

Disclosures: J.M. Barrett: None. **M.G. Raineri Tapies:** None. **G.M.G. Shepherd:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: E.04. Voluntary Movements

Support: UNAM-DGAPA-PAPIIT: IA201916, IA201018 to R-O, PE
CONACyT: FDC_1702 to R-O, PE
CONACyT: 463747 fellowship to P-F, AK

Title: Exploring the role of dorsolateral striatum in bimanually coordinated movements in rats

Authors: *A. K. PIMENTEL-FARFAN, A. BÁEZ-CORDERO, M. T. PEÑA-RANGEL, P. E. RUEDA-OROZCO;
Dept. de Neurobiologia del Desarrollo y Neurofisiologia, Inst. de Neurobiologia, UNAM, Queretaro, Mexico

Abstract: Many of our daily activities require bimanual movements with spatial and temporal coordination. This suggests that bilateral brain structures may work in synchrony to accurate execute such actions. In this context, the dorsolateral striatum (DLS) is anatomically privileged to integrate bilateral information to learn and perform skilled movements. DLS receives both bilateral and unilateral projections from sensorimotor cortices (MI/SI) and also from sensory thalamic regions such as the ventro-posterolateral nucleus (VPL). Therefore, we hypothesized that DLS uses bilateral sensorimotor inputs to coordinate bimanual outputs. To test this hypothesis, we designed a bimanual coordination task for rats where animals were trained to vertically and synchronously displace two levers (one with each forelimb) to get the reward. In this task, is possible to evaluate the onset/offset of bilaterally coordinated movements (a proxy of the beginning and end of the action) as well as the full movement trajectories of the forelimbs (a proxy of the execution of the action). Then we assessed the behavioral impact of unilateral lesions of the DLS or VLP. We found that with training, intact rats naturally developed coordinated forelimb movements, characterized by high correlation values between forelimb movements trajectories and by low interlimb onset/offset movement variability. In different groups of animals, unilateral DLS lesions performed before the beginning of the training significantly affected the correlation between forelimb movements but importantly, without affecting the interlimb onset/onset variability. Unilateral VPL lesions also decreased interlimb correlation but produced an increase in the interlimb onset/onset variability in many of the animals. Interestingly, similar lesions in the DLS performed after learning and overtraining, impacted behavior minimally. These results indicate that both DLS and VPL are indispensable for the development of bimanually coordinated movements. Ongoing experiments are focused on evaluating the role of bilateral sensorimotor cortical projections in this kind of movements.

Disclosures: A.K. Pimentel-Farfan: None. **A. Báez-Cordero:** None. **M.T. Peña-Rangel:** None. **P.E. Rueda-Orozco:** None.

Poster

064. Motor Systems: Fine Manual Control

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 064.24/N33

Topic: E.03. Basal Ganglia

Support: ZIA AA000416

Title: Dynamic engagement of sensorimotor interneurons during motor learning

Authors: *J. LEE[1], D. M. LOVINGER[2];
[1]NIAAA, Natl. Inst. of Hlth., Bethesda, MD; [2]Chief, Lab. Integrative Neurosci, Natl. Inst. on Alcohol Abuse and Alcoholism Rockville Office, Rockville, MD

Abstract: The acquisition and execution of motor skills are essential neural functions that play an important role in our daily lives. Circuitry in the primary motor cortex (M1), driven by excitatory projections and an inhibitory interneuron network, is thought to self-organize to facilitate motor planning, dexterous movement, and integration of sensory feedback. In recent years, the functional properties of excitatory and inhibitory neurons have been actively investigated through anatomical, physiological, and behavioral studies. However, the functional contribution of GABAergic interneurons in motor learning process remains unclear. Here, we focus on two key questions. From the 'disinhibition model' in which GABAergic interneurons across the neocortical network generally suppress excitatory transmission, a fundamental question arises as to whether consistent deactivation of GABAergic interneurons in M1 underlies vigorous and critical motor-output. A second question is whether the task-associated engagement of GABAergic interneurons would be stable or dynamic during a long-term learning process. To study these roles, we imaged somatostatin-expressing (SST) cortical interneurons in M1, a major class of inhibitory neurons in the cortex, while mice learned a self-paced voluntary lever-press for delivery of a food pellet reward and pellet retrieval movement. We used viral and transgenic strategies to express a genetically-encoded calcium indicator (GCaMP6s) in M1, and monitored calcium activity via a miniaturized head-mounted microscope in freely-moving mice over 4 weeks of training. Unlike previous observations of SST interneurons in the visual cortex, we found that population-level representation of SST in M1 was highly synchronized to the lever-press motion, in contrast to the 'disinhibition model'. Early training (within 1 week), the population-level synchronization of SST interneurons to lever-pressing was strengthened. Whereas in well-trained mice, these synchronized representations were significantly reduced, representing desynchronization and disengagement of single-neuronal responses during the same motor execution. In reversal learning tests, emergence of reproducible interneuron re-engagement was observed. Our results suggest that ensembles of motor cortex interneurons dynamically engage depending on the learning process and are supported by previous studies on

primate and rodent models that the primary motor cortex (M1) is preferentially active during learning new motor skills, but not in the execution of existing motor skills.

Disclosures: **J. Lee:** None. **D.M. Lovinger:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.01/N34

Topic: E.07. Rhythmic Motor Pattern Generation

Support: FRM DEQ20170336764

Title: Microglia shape the embryonic development of mammalian respiratory networks

Authors: ***M. THOBY BRISSON**[1], L. CARDOIT[1], M.-E. MAYEUR[2], O. PASCUAL[2]; [1]Univ. De Bordeaux, CNRS UMR 5287, Bordeaux, France; [2]CRNL-INSERM1028, Lyon, France

Abstract: Microglia, brain-resident macrophages, play key roles in shaping neural circuit function, and ensuring proper wiring and synapse homeostasis. Recent studies have also revealed an early function of these immune cells during prenatal nervous system development. Breathing rhythmogenesis arises from several interacting neural networks bilaterally distributed in specific regions of the brainstem. The initial assembly of these circuits occurs during embryonic development, although the role of microglia in this process remains unknown. Using a mouse line depleted for microglia (Pu.1 mutant mice) we addressed this issue by characterizing the breathing phenotype of Pu.1$^{-/-}$ mice *in vivo* and investigating the underlying cellular mechanisms with calcium imaging and electrophysiological recordings of neuronal activities *in vitro*. Pu.1$^{-/-}$ mice die at birth (if not earlier during embryonic development) at least partly due to a complete absence of breathing activity. Fictive respiratory activity recorded from isolated brainstems or slice preparations at different embryonic ages showed an abnormally slow frequency of the rhythmic activities generated by the embryonic parafacial respiratory group (epF) at the time of its functional onset (embryonic day 14, E14.5), by the preBötzinger complex (preBötC) at E15.5 and when both networks interact at E16.5. This deficit is associated with a reduced number of neurons active in the epF, leading to weaker and disorganized network activity, and defaults in the axon trajectories of commissural neurons that couple the bilateral preBötC half-centers. Coincidently, the anatomical detection of brainstem microglia, using Iba1 immunostaining or preparations obtained from Cx3CR1-GFP mice, showed an uneven distribution with a preferential presence at the locus of brainstem motor nuclei (hypoglossal, facial) and along the midline. Consistent with a general role of microglia in the regulation of axonal tract formation, neuronal migration and circuit wiring in different regions of the nervous system, our results

confer microglia with a crucial contribution to the proper establishment of the central respiratory command during embryonic development.

Disclosures: **M. Thoby Brisson:** None. **L. Cardoit:** None. **O. Pascual:** None. **M. Mayeur:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.02/N35

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grants NS72211
NSFC Young Scientists Fund 31701012

Title: Exploring the role of bombesin-related peptide-/peptide receptor-expressing neurons in formation of breathing pattern and sighs *in vivo*

Authors: ***Y. CUI**[1,2], D. N. CHIU[1], J. L. FELDMAN[1];
[1]Neurobio., UCLA, Los Angeles, CA; [2]Physiol., Chengdu Med. Col., Chengdu, China

Abstract: A simple microcircuit of parafacial (pF) neurons expressing the bombesin-related peptides neuromedin B (NMB) or gastrin releasing peptide (GRP) projecting to preBötzinger Complex (preBötC) neurons with cognate receptors generates signals that produce sighs, presumably critical for maintaining lung patency (Li et al., 2016). To understand the underlying mechanisms, we expressed channelrhodopsin (ChR2) in cells that express *Grp* (Grp-ChR2) or *Nmbr* (Nmbr-ChR2) by crossing Grp-Cre or Nmbr-Cre mice with floxed-ChR2-tdTomato mice (Ai27D); we also expressed ChR2 in preBötC GRPR neurons by injecting Flp-dependent ChR2 virus into preBötC of Grpr-Flp mice. In adult anesthetized Grp-ChR2 mice, photoactivation of pF GRP neurons with bilateral Long Pulse Photostimulation (LPP; 5-8 s pulse; 473 nm) was sufficient to induce ectopic sighs with a latency of ≥3 s from light onset, and increased breathing frequency, but did not change inspiratory burst amplitude. In contrast, LPP of preBötC *Grpr*- or *Nmbr*-expressing neurons: i) produced a sigh with a shorter delay than that resulting from activating pF GRP neurons (≥450 ms and ≥800 ms, respectively); and ii) increased the breathing frequency when targeting GRPR neurons, and augmented inspiratory bursts when targeting NMBR neurons. After blockade of both receptors with GRPR antagonist RC3095 and NMBR antagonist BIM23042, LPP of GRPR or NMBR neurons elicited biphasic bursts that resembled sighs in vagotomized mice and rats and fictive sighs recorded *in vitro*, indicating that initiating or increasing the activity of preBötC bombesin-related peptide receptor-expressing neurons is sufficient to generate biphasic, sigh-like bursts without activation of these receptors. We suggest that: i) GRP-GRPR or NMB-NMBR signaling in preBötC is not necessary for the generation of

sighs; ii) GRP and NMB affect sigh frequency and shape by activating their cognate receptor-expressing preBötC neurons; and iii) GRPR or NMBR neurons may activate, or be part of, distinct pathways in formation of breathing pattern and sighs.

Disclosures: Y. Cui: None. **D.N. Chiu:** None. **J.L. Feldman:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.03/N36

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH-NHLBI grant 1R35HL135779

Title: Network synchronization and synchrony propagation: Emergent elements of inspiration

Authors: *S. ASHHAD, J. L. FELDMAN;
Neurobio., UCLA, Los Angeles, CA

Abstract: While now well established that the preBötzinger Complex (preBötC) (Smith et al., 1991)is the kernel of breathing rhythmogenesis in mammals, the underlying mechanisms remain a mystery (Del Negro et al., 2018). We tested a novel (though non-exclusive) hypothesis, that the rhythm is an emergent property of the preBötC microcircuit. Specifically, we assessed preBötC network dynamics by recording synaptic inputs to inspiratory-modulated (I-M) somatostatin-expressing (SST^+) neurons under respiratory rhythmic and non-rhythmic conditions in *in vitro* slices from neonatal mouse. Under non-rhythmic conditions, these neurons received unsynchronized synaptic inputs that did not produce action potentials (APs). However, with increased neuronal excitability (by increasing bath [K^+]), their EPSPs transformed from sporadic to temporally clustered, producing more APs at shorter intervals. This increased activity percolated and grew slowly for ~250 ms, resulting in rhythmic population bursts. Wavelet analysis revealed that the power of these clustered inputs at 4- 64 Hz significantly increased in the epoch preceding, i.e., preinspiratory period, and during population bursts, but not immediately following bursts. Thus, our analysis revealed a dynamic reorganization of preBötC network activity correlated with and, we hypothesize, essential to, rhythmicity. In each cycle under normal conditions, an inspiratory burst (I-burst) emerges as a result of a transition of (presumptive) preBötC rhythmogenic neurons from aperiodic uncorrelated population spike activity to periodic synchronization during preinspiration; this activity subsides and the process repeats, resulting in a rhythm. preBötC SST^+ neurons were rarely connected through synaptic or electrical coupling, ruling out recurrent excitation as underlying synchrony. Yet, membrane potentials of simultaneously recorded I-M SST^+ pairs were strongly correlated at millisecond lags preceding and during inspiratory bursts, suggesting that convergent inputs underlie their

input synchrony. Strikingly, in a non-rhythmic slice, antagonizing $GABA_A$receptors could initiate this periodic synchronization and consequent rhythm, while simultaneously inducing a higher conductance state in preBötC output neurons. Appearance of such strong association between the network dynamics and neuronal properties to facilitate transfer via synchrony (Ratte et al., 2013) unveils a novel network mechanism for generation of inspiratory rhythm that emerges from the preBötC and propagates as synchrony to inspiratory motoneurons, driving inspiratory muscle contraction and consequential breathing movements.

Disclosures: S. Ashhad: None. **J.L. Feldman:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.04/N37

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Is Nav 1.6 sodium channel essential for generation of inspiratory activity *in vitro*?

Authors: *J.-C. VIEMARI[1], G. PITOLLAT[2], S. ZANELLA[3], F. BROCARD[3];
[1]CNRS-Institut de Neurosciences de la Timone, Marseille, France; [2]Aix-Marseille Univ., Marseille, France; [3]Inst. de Neurosciences de la Timone, Marseille, France

Abstract: The network responsible for the central command of breathing is embedded in the ventral respiratory column (VRC) of the brainstem. Within the VRC, the first described respiratory oscillator, the preBotzinger Complex (preBotC), is both necessary and sufficient to generate inspiratory activity. Two other oscillators have been described, the Post-inspiratory Complex (PiCo) for the control of post-inspiration and the lateral parafacial nucleus (pFL), for the control of active expiration.
Inspiratory activity generated within the preBtoC relies on pacemaker properties that depends on the persistent sodium current (I_{NaP}) and the cationic non-selective current (I_{CAN}). I_{NaP} and pacemaker activity have been postulated to be essential for inspiratory rhythm generation and have been incorporated in models of respiratory neural circuits. However, the channels responsible for the persistent sodium current remain to be determined. In CNS neurons, several sodium channel subunit mRNAs are expressed, including Nav1.1, Nav1.2, Nav 1.3, Nav1.5 and Nav1.6. In tissue samples from the preBotC and rVRG regions, only Nav1.1, Nav1.2, Nav 1.3 and Nav1.6 mRNAs were detected (Ptak et al, 2005) and Nav 1.1 and Nav 1.6 seemed predominantly expressed. Here, we investigated the role of Nav 1.6 through the used of Nav 1.6 null mutant mice where the amplitude of the persistent sodium current is reduced. We investigated the effects of the null mutation on the inspiratory rhythm generation and on the hypoxic response. Although, the inspiratory rhythm is not significantly affected in Nav1.6 null mutation, blockade of I_{CAN} with FFA 40-80 µM suggested an important role for other Nav

isoforms. *In vivo,* plethysmography recordings also showed an affected response to hypoxia in Nav 1.6 null mutants.
Taken together, Nav 1.6 is involved in the regulation of the hypoxic response *in vivo* but do not seem to play an essential role in the inspiratory rhythm generation eventhough compensatory mechanisms can not be excluded in the Nav 1.6 null mutant.

Disclosures: J. Viemari: None. **G. Pitollat:** None. **S. Zanella:** None. **F. Brocard:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

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Program #/Poster #: 065.05/N38

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01HL079294

Title: The role of TRP channels in the pre-Botzinger complex inspiratory rhythm and breathing

Authors: *A. K. TRYBA;
Pediatric Neurol., The Univ. of Chicago, Chicago, IL

Abstract: Inspiratory breathing rhythms generated by the medullary pre-Botzinger Complex (pre-BotC) neural network play a critical role in gas exchange that sustains life. The neural mechanisms that govern the respiratory rhythm and pattern, including the cellular/ionic mechanisms and specific neurons of the respiratory control center are not fully known. We hypothesize that cycle by cycle reliability of the eupneic inspiratory rhythm depends on synaptic activation of intrinsic membrane currents, carried by TRP channels. Glutamatergic synaptic drive together with excitatory neuromodulators may activate TRPC3/7 and/or TRPM4 channels that are proposed to underlie or enhancing rhythmic inspiratory activity. We tested if TRPC3/7 channels and/or TRPM4 channels play a role in inspiratory rhythms using mouse brain slices containing the preBotC network that generates inspiratory rhythms *in vitro*. Antagonists blocking either TRPC3/7 or TRPM4 channels alone did not eliminate the rhythm, but blocking both TRPC3/7 and TRPM4 led to irregular, less frequent and cessation of inspiratory rhythms; suggesting both TRPC3/7 and TRPM4 play a role in inspiratory rhythms (n=5). To further test the role of TRPC3/7, in rhythmic *in vitro* respiratory brain slices, intracellular recordings were made that dialyzed preBotC inspiratory neurons with antibodies (Ab) that block TRPC3/7 activity; applying Ab significantly reduced inspiratory neuron drive potentials and bursting properties (n=6). Bath applied TRPC3 activator (GSK1702934A) also enhances rhythmic inspiratory network bursting *in vitro* (n=4). *In vivo* respiratory plethysmography and *in vitro* brain slices of $TRPC3^{-/-}$ mice additionally revealed irregular, slow inspiratory rhythms (n=4). To examine the role of TRPC3/7 channels in breathing *in vivo,* siRNA that selectively knocks down

expression of TRPC3/7 channels in neurons was stereotaxically injected into the preBotC in rats. Using siRNA, reducing neuronal expression of TRPC3/7 within the pre-BotC region results in repetitive sleep apneas (n=7/7). Our data suggest TRPC3/7 channels play a critical role in breathing, particularly during sleep.

Disclosures: A.K. Tryba: None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.06/N39

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01NS073875
GSU B & B program

Title: Morphine induced respiratory depression: Behavioral, phrenic and brainstem respiratory neuronal evidence

Authors: *H. XING[1], C. M. JOHNSON[2], N. SABATE[1], C. JIANG[1];
[2]Biol., [1]Georgia State Univ., Atlanta, GA

Abstract: A major adverse effect of the chronic use of opioid drugs is respiratory depression causing a loss of a large number of lives each year. To address the question as to how chronic morphine exposures affect brainstem respiratory motor output, we performed these studies in Sprague Dawley rats (7-week, either genders), using repetitive morphine administrations (10mg/kg, *ip*, twice a day for consecutive 6 days). After the chronic treatments, the same dose morphine suppressed the minute ventilation by 55.2% ± 4.1 (n=16 rats) for 3-4 h accompanied with severe variations in tidal volume and breathing frequency as measured in plethysmography. Such effects reached plateau levels in 3-4 days. Recording from these rats in spontaneous breathing after decerebration, we found similar changes in breathing activity in phrenic discharges. Unexpectedly, ectopic phrenic activity during expiration was seen in these rats after chronic morphine exposures. Firing activity of respiratory neurons was recorded extracellularly in the ventral respiratory group. A large number of E-I phase-spanning neurons were observed in rats treated with chronic morphine but not with saline injection. These results suggest that the respiratory depression after chronic morphine exposures does not seem due to general suppression of brainstem respiratory motor output solely, while corruptions in rhythmic regulation of the motor output may play a more important role.

Disclosures: H. Xing: None. **C.M. Johnson:** None. **N. Sabate:** None. **C. Jiang:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.07/N40

Topic: E.07. Rhythmic Motor Pattern Generation

Support: American Heart Association

Title: Intermittent hypoxia causes cardiometabolic dysfunction in obese, ob/ob mice

Authors: *S. N. FRAMNES-DEBOER, A. A. JONES, D. M. ARBLE;
Dept. of Biol. Sci., Marquette Univ., Milwaukee, WI

Abstract: Obstructive sleep apnea (OSA) is a common sleep disorder characterized by repeated bouts of intermittent hypoxia (IH). Approximately, 40-70% of the obese population exhibit OSA and are at higher risk for developing hypertension, heart failure, and other cardiometabolic diseases. Leptin, a hormone increased with obesity, is also associated with cardiometabolic disease. It is unclear if it is leptin insensitivity or other aspects of obesity that make individuals more susceptible to cardiometabolic diseases when experiencing IH in the form of OSA. To determine the relative involvement of leptin and/or obesity in the cardiometabolic outcomes of IH, we exposed lean C57Bl/6J WT mice, leptin-deficient *ob/ob* mice, and weight-restricted *ob/ob* mice to 6 days of IH (a 30-second, 5% O_2 desaturation event occurring every 6 minutes for 9 hours/day). We found that IH led to a negative energy balance in WT mice, resulting in both a decrease in food intake and weight loss. Interestingly, while leptin-deficient *ob/ob* mice also lost weight, these mice exhibited an increase in feeding with the IH exposure. These data suggest that leptin signaling during IH serves to reduce food intake. We further found that the obese, leptin-deficient *ob/ob* mice exhibited an IH-induced impairment in cardiac function as indicated by an increase in left ventricle internal diameter diastole and an increase in cardiac output. These cardiac impairments were not observed in either the WT or the weight-restricted *ob/ob* group. Taken together, we find that a 6-day exposure of IH is associated with a negative energy balance in mice and that both impaired leptin signaling and obesity are necessary for IH-induced cardiac impairments. Further research will focus on food intake and how that may mitigate cardiometabolic disease in OSA patients.

Disclosures: S.N. Framnes-DeBoer: None. **A.A. Jones:** None. **D.M. Arble:** A. Employment/Salary (full or part-time):; Marquette University.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.08/N41

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH NINDS Grant 1R01NS107421-01

Title: Naked mole rats: New model to investigate the central respiratory network *in vitro*

Authors: J.-C. VIEMARI[1], B. M. BROWE[2], T. J. PARK[3], ***A. J. GARCIA, III**[4];
[1]CNRS-Institut de Neurosciences de La Timone, Marseille, France; [2]Biol. Sciences- Neurobio., [3]Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL; [4]The Univ. of Chicago, Chicago, IL

Abstract: The naked mole rat (NMR) is a unique mammalian species that is long lived and extremely resistant to hypoxia. Hypoxia resistance in the NMR, is due in part, to the unique ability for this species to employ fructose-driven glycolysis. Based on these previous findings, we hypothesized that changes in oxygenation would not impact the frequency of rhythmogenesis in the *en bloc* preparation of the NMR. Recordings were made from the ventral respiratory column (VRC), at the level of the preBötzinger complex (preBötC), and/or motor output from the C4 ventral root. Inspiratory rhythms could be recorded in *en bloc* preparations from animals as old as P44. Rhythmogenesis during hypoxia could be generated in both glucose and fructose media suggesting that the respiratory network can employ fructose-driven glycolysis for rhythm generation. Additionally, unique changes in tissue oxygen were observed during hypoxia and reoxygenation suggesting dramatic changes in oxygen consumption. Ongoing experiments show that the frequency of central inspiratory rhythmogenesis is responsive to hypoxia, suggesting that the central rhythm generation is sensitive to changes in oxygenation despite the unique properties of the NMR in hypoxia. Our findings suggest that the NMR represents a powerful model to investigate the neural basis of respiratory control in long lived laboratory animals resistant to hypoxia. This work may lead to new insights into mechanisms conferring hypoxia tolerance during conditions such as stroke, cardiac arrest and head trauma.

Disclosures: **J. Viemari:** None. **B.M. Browe:** None. **T.J. Park:** None. **A.J. Garcia:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.09/N42

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant NS72211

Title: Chemogenetic exploration of the role of Somatostatin and Neuromedin B receptor-expressing neurons in the formation of breathing pattern and sighs *in vivo*

Authors: *E. BONDARENKO, R. ABREU, J. L. FELDMAN;
Neurobio., UCLA, Los Angeles, CA

Abstract: Somatostatin-positive (SST^+) neurons within the preBötzinger complex (preBötC) are critical for producing breathing pattern, but not rhythmogenesis (Cui et al., 2016). Parafacial neurons produce peptides Neuromedin B (NMB) and Gastrin Releasing Peptide (GRP) that act on preBötC neurons with cognate receptors to evoke sighs (Li et al., 2016). Here we explore effects of selectively activating and inhibiting two subpopulations of preBötC glutamatergic neurons using DREADDs in two Cre-mouse lines: SST^+neurons and neurons that express receptors for NMB ($NMBR^+$). In anesthetized mice, microinjection of NMB+GRP into preBötC increased amplitude and reduced frequency of breathing, and also evoked sigh-like "doublets" (two breaths occurring immediately after one another). Using stereotaxic delivery of Cre-dependent AAVs, we selectively expressed excitatory hM3Dq or inhibitory hM4Di receptors in SST^+or $NMBR^+$preBötC neurons. Exciting SST^+neurons with Clozapine-N-Oxide (CNO) increased sighing frequency in awake mice and induced "doublets" in anesthetized mice. Inhibiting SST^+neurons with CNO blocked NMB+GRP-mediated production of "doublets" but did not affect basal breathing. Exciting $NMBR^+$neurons with CNO increased amplitude, reduced frequency of breathing, but did not induce sighing in awake or anesthetized mice. We propose that sighing requires two separate mechanisms. One mechanism is mediated by preBötC $NMBR^+$neurons that induces increased breathing amplitude characteristic of sighs. A second mechanism involving preBötC SST^+neuron modulates breathing timing to produce a sigh(-like) breath, presumably with involvement of GRP.

Disclosures: E. Bondarenko: None. **R. Abreu:** None. **J.L. Feldman:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.10/N43

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

Support: NIH grant R01NS085227

Title: Pou3f1 is required for the identity and function of inspiratory motor neurons in the developing spinal cord

Authors: *S. KIM[1], A. Y. HAN[1], K. KAM[1,3], J. L. FELDMAN[1], B. G. NOVITCH[1,2]; [1]Neurobio., [2]Eli and Edythe Broad Ctr. of Regenerative Med. and Stem Cell Res., UCLA, Los Angeles, CA; [3]Cell Biol. and Anat., Chicago Med. Sch., Chicago, IL

Abstract: Respiration is an essential motor behavior, required from birth to death in all terrestrial species. Respiratory movements are driven by the alternating functions of inspiratory and expiratory motor neurons (MNs) located in the spinal cord, which receive both direct and indirect premotor inputs from rhythm generating neurons located in the brainstem. The mechanisms accounting for the diversity of respiratory MN subtypes and selective receipt of inspiratory vs. expiratory premotor inputs remain unclear. Here, we examine the functions of two transcription factors, Pou3f1 and Bcl11b, that are respectively associated with formation of inspiratory and expiratory MNs. In the absence of Pou3f1, both phrenic and external (inspiratory) intercostal MN numbers are reduced and the remaining cells are abnormally dispersed within the ventral horn of the spinal cord. Pou3f1 loss is further associated with decreased outgrowth of the phrenic nerve, a lack of distal innervation of the diaphragm, reductions in respiratory premotor synapses, and disruptions in rhythmic inspiratory motor activities. Bcl11b loss, on the other hand, leads to aberrant expression of Pou3f1 and disruptions in respiratory motor axon projections. Together, these data identify new functional regulators of respiratory motor neuron identity and suggest the deployment of distinct transcriptional programs for inspiratory and expiratory motor functions.

Disclosures: S. Kim: None. **A.Y. Han:** None. **K. Kam:** None. **J.L. Feldman:** None. **B.G. Novitch:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.11/N44

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R01 HL104127

Title: Role of synaptic inhibition in the coupling of the respiratory rhythms that underlie eupnea and sigh behaviors

Authors: *D. S. BORRUS[1], G. D. CONRADI SMITH[2], C. A. DEL NEGRO[3];
[1]Applied Sci., Col. of William and Mary, Williamsburg, VA; [2]The Col. of William & Mary, Williamsburg, VA; [3]Dept. of Applied Sci., William & Mary, Williamsburg, VA

Abstract: The preBötzinger Complex (preBötC) is the origin for two distinct rhythms that drive breathing movements underlying eupnea and sighing. Eupnea refers to periodic inspiratory breaths that ventilate the lungs. Sighs occur at a much lower frequency. They serve to reinflate collapsing alveoli and are essential for optimal pulmonary function. Here, we examine the neural mechanisms in the preBötC that couple eupnea- and sigh-related rhythms. A sigh typically manifests quickly after - seemingly initiated by - a eupneic breath. It has been proposed that chloride-mediated synaptic inhibition couples the sigh to a preceding eupneic breath. Following the sigh, the next eupneic breath is delayed for almost an entire additional inspiratory cycle, which could also reflect synaptic inhibition linking the sigh to the eupnea rhythm. This study examines the potential role(s) of chloride-mediated synaptic inhibition in preBötC rhythms using neonatal mouse brainstem slice preparations. First, we show previously undocumented variability in the temporal relationship between sigh bursts and their preceding eupnea-related inspiratory bursts. Next, we selectively block glycinergic and ionotropic GABAergic synapses, which surprisingly strengthens (rather than weakens) the phasic relationship between sigh bursts and inspiratory bursts. Further, disinhibition does not alter the prolonged interval following a sigh prior to resumption of the inspiratory rhythm. These results demonstrate that coupling between the two breathing rhythms does not depend on chloride-mediated synaptic inhibition.

Disclosures: D.S. Borrus: None. **G.D. Conradi Smith:** None. **C.A. Del Negro:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.12/N45

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Macquarie University Scholarship

Title: Respiratory sympathetic coupling mechanism: Comparative role of post-inspiratory neurons in post-inspiratory complex (PICO) & kolliker-fuse (KF) nucleus

Authors: *R. TOOR, Q.-J. SUN, S. MCMULLAN, C. HILDRETH, J. PHILLIPS;
Biomed. Sci., Macquarie Univ., Sydney, Australia

Abstract: Enhanced respiratory-sympathetic coupling, mediated by post-inspiratory and sympathetic neurons, may lead to hypertension via changes in mechanisms controlling vascular resistance. The post-inspiratory neuronal population that is critical for coupling is not known. The present study aimed to investigate the potential role of post-inspiratory neurons in the post-inspiratory complex (*PiCo*) in the brainstem and those in the Kolliker-Fuse (*KF*) nucleus in the pons. We made microinjection of the GABA agonist isoguavacine (10 mM) into *PiCo* or *KF* while simultaneously recordings from phrenic, vagus and renal sympathetic nerves in anaesthetised, vagotomised, paralysed and artificially ventilated adult Lewis rats. The nerve responses were compared before and after bilateral inhibition under normotensive conditions and during hypoxia. The amplitude of the post-inspiratory peak both on vagus and sympathetic nerves was measured along with other respiratory parameters to analyze post-I interaction and coupling patterns respectively. Bilateral Inhibition of *PiCo* reduced, but did not eliminate the post-inspiratory peak both on vagus (n=9, 3.9 ± 0.5 to 1.4 ± 0.3 μV, P< 0.01) and renal sympathetic nerve (n=9, 4.2 ± 0.7 to 2.1 ± 0.4 μV, P< 0.01) with slight increase in respiratory frequency. Hypoxia trial immediately after *PiCo* inhibition restored post-I peak, both on vagus and sympathetic nerve. In contrast, inhibition of KF eliminated the post-I peak (n=9, 4.19 ± 0.60 to 0.75 ± 0.09 μV P< 0.0007) with a simultaneous, parallel and comparable reduction of the post-I peak on the renal sympathetic nerve (n=9, 3.19 ± 0.54 to 0.73 ± 0.16 μV P< 0.0008). KF inhibition also blocked the vagal and sympathetic nerve post-I increase to hypoxia. The findings suggest, the neurons in *the PiCo* contribute to coupling and the neurons in the KF are critical for post-I coupling and play a decisive role in respiratory-sympathetic modulation.

Disclosures: R. Toor: None. **Q. Sun:** None. **S. McMullan:** None. **C. Hildreth:** None. **J. Phillips:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.13/N46

Topic: E.07. Rhythmic Motor Pattern Generation

Support: HL 104127

Title: Fourier analysis applied to time series calcium imaging in the preBötzinger complex can delineate constituent dbx1 derived rhythmically active interneurons

Authors: *C. J. GROVER, C. A. DEL NEGRO;
Dept. of Applied Sci., William & Mary, Williamsburg, VA

Abstract: The respiratory central pattern generator (CPG) for the inspiratory breathing movements is contained in the preBötzinger complex (preBötC) of the lower medulla. Rhythmic activity of its constituent interneurons can be investigated using slice models of breathing that isolate the preBötC yet retain the ability to generate rhythm and rudimentary motor output in vitro. To monitor multiple preBötC neurons simultaneously, we employ two-photon microscopy in conjunction with slices from mice that express genetically encoded calcium indicators. GCaMP6f, the genetically encoded calcium indicator we favor, does not express in cell nuclei, which greatly complicates conventional automated cell detection routines. Here we describe an analysis routine, implemented in MATLAB, that delineates preBötC interneurons from time series GCaMP6f fluorescence data. The algorithm utilizes Fourier analysis to identify groups of contiguous pixels whose frequency of rhythmic calcium transients matches the frequency of ensemble activity in the preBötC network (without a priori expectations of cell shape). Because constituent neurons are selected based on their frequency and activity pattern matching overall network activity, we content that this algorithm could be applied to identify constituent neurons of any CPG, regardless of frequency of calcium transients, constituent neuron morphology, or expression pattern of the calcium indicator.

Disclosures: C.J. Grover: None. **C.A. Del Negro:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.14/O1

Topic: E.07. Rhythmic Motor Pattern Generation

Support: National Institutes of Health grants NS07221, HL135779, and NS097492
Swedish Research Council (Vetenskapsrådet)

Title: Inspiratory rhythmogenic activity in preBötzinger complex is burst-independent and opioid-sensitive

Authors: *C. THÖRN PEREZ[1], X. SUN[1], N. HALEMANI[2], X. M. SHAO[3], M. GREENWOOD[4], S. HEATH[1], J. L. FELDMAN[1], K. KAM[2];
[1]Neurobio., UCLA, Los Angeles, CA; [2]Cell Biol. and Anat., Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL; [3]Neurobio., David Geffen Sch. Med. at UCLA, Los Angeles, CA; [4]Rosalind Franklin Univ. of Med. and Sci., Chicago, IL

Abstract: Stereotyped movements underlie basic behaviors like locomotion and vital functions like breathing. Rhythmic activity is generated by neural circuits known as central pattern generators; although, mechanisms for rhythm generation remain unknown. The mammalian respiratory rhythm is unique in that a region critical for rhythmogenesis, the preBötzinger Complex (preBötC), is localized. preBötC population activity points to each cycle being initiated by a subpopulation of neurons that generate rhythmic burstlets. With sufficient recurrent excitation, preBötC neurons generate an inspiratory burst. We hypothesize that burstlets constitute the rhythmogenic core driving inspiration. Neuromodulators, such as substance P and opioids, can affect burst frequency. Here, we show in rhythmic medullary slices containing the preBötC from neonatal mice that the μ-opioid receptor (μOR) agonist [D-Ala^2,N-MePhe^4,Gly-ol^5]-enkephalin (DAMGO), which decreases inspiratory burst frequency, also decreased burstlet frequency, supporting the hypothesis that burstlets drive the breathing rhythm. Moreover, the effect of DAMGO was reduced by Substance P, but not by blockade of inhibitory synaptic transmission, suggesting that neuropeptides like opioids and Substance P act directly on rhythmogenic neurons while inhibitory effects occur upstream of μOR signaling in the preBötC or in downstream pattern generating populations. Furthermore, with the use of advanced fluorescence in situ hybridization techniques (RNAscope), we show that μORs are present on a subpopulation of rhythmogenic preBötC neurons. When μORs are genetically deleted from this population (*Dbx1^{Cre}; Oprm1$^{fl/fl}$*), DAMGO no longer affects burst frequency.
The effects of preBötC μOR activation contribute to our understanding of the general principles of neural circuit function in mammals and may yield strategies for alleviating opioid-induced depression of breathing.

Disclosures: C. Thörn Perez: None. **X. Sun:** None. **N. Halemani:** None. **X.M. Shao:** None. **M. Greenwood:** None. **S. Heath:** None. **J.L. Feldman:** None. **K. Kam:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.15/O2

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R01 HL104127 (PI: Del Negro)

Title: Burstlet hypothesis of inspiratory rhythm generation: Are rhythm- and pattern- generation separate mechanisms?

Authors: *P. KALLURKAR, C. GROVER, M. C. PICARDO, C. A. DEL NEGRO;
Applied Sci., William & Mary, Williamsburg, VA

Abstract: Breathing is a vital rhythmic behavior, which emanates from a central pattern generator (CPG) network. The respiratory core oscillator is contained in the preBötzinger complex (preBötC) of the lower brainstem, where excitatory (glutamatergic) interneurons generate the neural rhythm and basic motor pattern for inspiration in mammals. Here we aim to differentiate the mechanisms underlying rhythm and pattern in medullary slices from newborn mice that isolate the preBötC and remain rhythmically active with inspiratory motor output measurable from the hypoglossal nerve (XII). Specifically, we test 'burstlet' theory (Kam *et al. J Neurosci* 33: 9235, 2013), which posits that low amplitude burstlets, subthreshold from the standpoint of inspiratory bursts and XII motor output, reflect the fundamental oscillator of the preBötC. In turn, a discrete suprathreshold process transforms burstlets into full amplitude inspiratory bursts that drive XII motor output. Our results largely recap features identified by Kam & Feldman: preBötC neural activity consist of bursts and concurrent XII motor output, intermingled with lower amplitude preBötC burstlets, that do not produce XII motor output. Nevertheless, under most circumstances, burstlets form the preinspiratory phase of full amplitude inspiratory bursts in the preBötC. Building on results by Kam *et al.*, we found that burst and burstlet rhythms are both voltage-dependent; graded changes in external K^+ concentration regulate the frequency of bursts and burstlets. We also detected burstlets in whole-cell recordings of single preBötC rhythmogenic neurons and in photonic recordings of subpopulations of preBötC rhythmogenic neurons. We conclude that burstlet theory is viable explanation for inspiratory rhythmogenesis.

Disclosures: P. Kallurkar: None. **C. Grover:** None. **M.C. Picardo:** None. **C.A. Del Negro:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.16/O3

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF award DMS 1612913
CRCNS award DMS 1724240

Title: Effects of persistent sodium current blockade in respiratory circuits depend on the pharmacological mechanism of action and network dynamics

Authors: *R. S. PHILLIPS, J. E. RUBIN;
Mathematics, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The mechanism(s) of action of most commonly used pharmacological blockers of voltage-gated ion channels are well understood; however, this knowledge is rarely considered when interpreting experimental data. Effects of blockade are often assumed to be equivalent, regardless of the mechanism of the blocker involved. Using computer simulations, we demonstrate that this assumption may not always be correct. We simulate the blockade of a persistent sodium current (I_{NaP}), proposed to underlie rhythm generation in pre-Bötzinger complex (preBötC) respiratory neurons, via two distinct pharmacological mechanisms: (1) pore obstruction mediated by tetrodotoxin and (2) altered inactivation dynamics mediated by riluzole. The reported effects of experimental application of tetrodotoxin and riluzole in respiratory circuits are diverse and seemingly contradictory and have led to considerable debate within the field as to the specific role of I_{NaP} in respiratory circuits. The results of our simulations match a wide array of experimental data spanning from the level of isolated preBötC neurons to the level of the intact respiratory network and also generate a series of experimentally testable predictions. Specifically, in this study we: (1) provide a mechanistic explanation for seemingly contradictory experimental results from *in vitro* studies of I_{NaP} block, (2) show that the effects of I_{NaP} block in *in vitro* preparations are not necessarily equivalent to those in more intact preparations, (3) demonstrate and explain why riluzole application may fail to effectively block I_{NaP} in the intact respiratory network, and (4) derive the prediction that effective block of I_{NaP} by low concentration tetrodotoxin will stop respiratory rhythm generation in the intact respiratory network. These simulations support a critical role for I_{NaP} in respiratory rhythmogenesis *in vivo* and illustrate the importance of considering mechanism when interpreting and simulating data relating to pharmacological blockade.

Disclosures: R.S. Phillips: None. **J.E. Rubin:** None.

Poster

065. Neuronal Analysis of Respiratory Networks

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 065.17/O4

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Fundación Beltrán-Morgado para el Avance y Difusión de la Neurociencia en Veracruz

Title: *In vitro* sighs in rat are inspiratory bursts with longer duration rather than larger amplitude

Authors: *C. MORGADO-VALLE, L. LOPEZ-MERAZ, C. A. PEREZ-ESTUDILLO, L. BELTRAN-PARRAZAL;
Ctr. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, VER, Mexico

Abstract: The preBötzinger Complex (preBötC) is a small area in the ventrolateral medulla necessary and sufficient for generation of the inspiratory phase of the respiratory rhythm. Evidence shows that, although the preBötC is not a chemosensitive nucleus, it is susceptible to modulation from chemosensitive areas such as the nucleus of the solitary tract (NTS) and the retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG). This modulation leads to the emergence of distinctive respiratory patterns such as sighing. *In vitro,* sighing has been studied in mice, using the brainstem transverse slice that includes the preBötC and the XII nerve (XIIn) as motor output. Here, we aimed to characterize sighs in the rat transverse slice preparation. We evoked sighs by bath applying the peptides bombesin or substance P (SP) while recording both the integrated XIIn (∫XIIn) motor activity and the inspiratory drive from preBötC neurons. We found that in rat, *in vitro* sighs occur in basal conditions every 36.8±5.3 s, whereas in the presence of bombesin every 16.6±3 s and of SP every 23±6 s. In recordings from both ∫XIIn and preBötC inspiratory neurons, sighs were better described as bursts with longer duration rather than larger amplitude. We did not find statistically significant differences in the duration of the after-sigh interburst interval (IBI) compared with the IBI after non-sighs. Bombesin and SP increased the frequency of respiratory rhythm and therefore, the frequency of sighs. These results suggest that the signal cascades activated by bombesin and SP to evoke sighing are related to the mechanisms regulating burst duration.

Disclosures: C. Morgado-Valle: None. **L. Lopez-Meraz:** None. **C.A. Perez-Estudillo:** None. **L. Beltran-Parrazal:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.01/O5

Topic: E.09. Motor Neurons and Muscle

Title: Temporal profile of transient corticomotoneuronal direct connection during development in the rodent

Authors: *T. OHNO[1], S. FUKUDA[2], N. MURABE[3], H. MIZUKAMI[5], K. OZAWA[5], T. HAYASHI[4], M. SAKURAI[6];
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Abstract: Monosynaptic corticomotoneuronal (CM) connections that are characteristic of higher primates play an important role in the cortical control of skilled hand movements. We recently demonstrated direct connections between the corticospinal axons and cervical motoneurons (MNs) innervating forearm muscles in the juvenile rodent (Maeda et al, 2016). We further showed that those CM direct connections were eliminated until adulthood using the technique of retrograde trans-synaptic labeling with genetically-modified rabies virus (Murabe et al, 2018). However, accurate elimination-time-course of the transient direct connections and the underlying mechanisms of regression process still remain unclear because of the technical difficulties in recording EPSCs from the spinal MNs after postnatal two weeks. Because the spinal MNs are known to be vulnerable in slices, studies of synaptic connections in MNs were seriously limited in mature animals. In this study, we devised a new slice preparation technique for improving viability of spinal cord slices, which allowed us to make whole cell recordings from the spinal MNs in young adult mice (up to seven-weeks-old). Utilizing this method, we examined the proportion of monosynaptic corticospinal tract (CST)-MN-EPSCs from postnatal day (P) 6 to P50 MNs innervating forelimb muscles. Positive ratio of monosynaptic CST-MN-EPSCs increased from P6 to P8, when it approached plateau (P6: 38.1% (n=21), P7: 50.0% (n=12), P8: 66.7% (n=27)). The plateau phase lasted from P8 to P13 (P8: 66.7% (n=27), P9: 64.7% (n=17), P10-11: 66.7% (n=12), P12-13: 63.6% (n=11)). The positive ratio began to decrease from P14, and then disappeared at P22 (P14-15: 45.5% (n=11), P16-17: 40.0% (n=10), P18-19: 27.3% (n=22), P20-21: 4.5% (n=22), P22-29: 0% (n=12), P30-50: 0% (n=10)). On the other hand, even after P21, monosynaptic Ia-EPSCs were recorded from spinal MNs and CST-EPSCs from spinal interneurons indicating that CST or MNs themselves are intact. These results indicate that there are transient direct synaptic connections between CST and the spinal MNs during early development that disappear by P22. This elimination time resided within the range (P10-P22) estimated by our previous study using retrograde trans-synaptic labeling.

Disclosures: **T. Ohno:** None. **S. Fukuda:** None. **N. Murabe:** None. **H. Mizukami:** None. **K. Ozawa:** None. **T. Hayashi:** None. **M. Sakurai:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.02/O6

Topic: E.09. Motor Neurons and Muscle

Support: CNRS
Bourse ministérielle de l'enseignement supérieur français

Title: Impact of a motor training on the maturation of the mouse lumbar spinal cord

Authors: *C. QUILGARS[1], G. COURTAND[2], L. CARDOIT[2], P. DE DEURWAERDÈRE[2], F. E. PERRIN[3], S. S. BERTRAND[2];
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Abstract: Neuronal activity has been shown to be essential for the proper formation of neuronal circuits. In spinal neuronal networks, spontaneous activity triggers spontaneous limb movements, which are visible in both vertebrate embryos and newborns. It is now well admitted that the activation of the sensorimotor loop induced by these spontaneous activities/movements plays a major role in the maturation and refinement of spinal motor networks during development. However, the cellular bases of the developmental changes induced by activity are, so far, largely ignored in mammals. To bridge this gap, we used a motor training as a tool to increase the activity in immature spinal motor networks at birth and deciphered the induced cellular changes. Newborn mice (1 to 2 days old) were trained to swim twice a day and the impact of motor training was assessed in 3 days old mice (1) at the behavioral level by studying the development of the swimming motor pattern, (2) on the membrane properties of lumbar motoneurons recorded intracellularly in spinal cord slices, (3) on the spontaneous synaptic inputs received by motoneurons, (4) on the activity-dependent synaptic plasticity of the reticulospinal connections impinging onto motoneurons and (5) on the invasion of the spinal circuitry by descending monoaminergic fibers using a chromatographic approach.
Our preliminary data show that motor training triggered an accelerated maturation of spinal motor networks characterized by a precocious acquisition of the 4 limbs swimming motor pattern in trained animals. These behavioral modifications were coupled with changes in motoneuron membrane properties, synaptic plasticity and synaptic input modulation. Alterations in spinal moaminergic systems were also found in the spinal cord of trained animals.
Altogether these results demonstrate that a brief motor training performed just after birth in newborn mice is able to impact the maturation of spinal motor networks. The comparison of the

myosin composition of hindlimb muscles as well as the expression of trophic factors in the spinal tissue of control and trained animals are in progress.

Disclosures: C. Quilgars: None. **G. Courtand:** None. **L. Cardoit:** None. **P. De Deurwaerdère:** None. **F.E. Perrin:** None. **S.S. Bertrand:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.03/O7

Topic: E.09. Motor Neurons and Muscle

Support: AFM 21041

Title: Target specific modulation of synaptic transmission from spinal motoneurons by muscarinic receptors

Authors: A. MOHAMED LAFIRDEEN, E. TOKARSKI, P. ASCHER, ***B. LAMOTTE D'INCAMPS**;
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Abstract: In addition to the muscles, Motoneurons (MNs) contact several intraspinal targets, and thus participate in the recurrent control of their activity. Their best known spinal targets are the Renshaw cells (RCs), a population of inhibitory interneurons that themselves inhibit the synergistic MNs. In the mouse spinal cord, MNs also contact neighboring MNs[1], as well as V3 interneurons[2], a population of excitatory interneurons that participate in motor control. At the neuromuscular junctions (NMJs), the synaptic transmission is mediated by the release of acetylcholine (ACh). At the MN-RC synapse, two neurotransmitters are coreleased (ACh and glutamate) and activate independently their postsynaptic receptors[3]. Finally, neighboring MNs and V3 interneurons are excited by postsynaptic currents mediated by glutamate receptors[1,2]. At the neuromuscular junction, muscarinic receptors produce presynaptic effects via a complex interaction between m1 and m2 receptors[4, 5]. Here we have examined the effects of muscarinic agonists on two intraspinal targets of the MNs, and found that muscarinic modulation of the synaptic strength occurs at the MN-RC synapse. Activation of mAChRs by the bath-application of muscarine (10 µM) induces a reduction by 50 % ± 10 % of the recurrent EPSC recorded in RCs (n=13). This inhibition vanishes when m2 receptors are blocked by methoctramine (1 µM). In contrast the synaptic transmission from MNs towards neighbhouring MNs (which is mediated by the release of glutamate[1]) appears unaffected by m2 mAChRs. This suggests that the muscarinic modulation of synaptic transmission is specifically affecting the synapses releasing ACh - the NMJs and those contacting the RCs - and not those contacting the MNs. Our experiments thus suggest that mAChRs, are only activated at the synapses at which ACh has

been shown to be released, and in which ACh could modulate its own release. The mAChRs involved could be located on the axon terminals or on neighboring glial cells as seen at the NMJ[6, 7].

1. G. S. Bhumbra, M. Beato, *PLoS Biol.* **16**, e2003586 (2018), doi:10.1371/journal.pbio.2003586.
2. J. W. Chopek *et al.*, *Cell Rep.* **25**, 146-156.e3 (2018), doi:10.1016/j.celrep.2018.08.095.
3. B. Lamotte d'Incamps *et al.*, *Sci. Rep.* **7**, 4037 (2017), doi:10.1038/s41598-017-04266-8.
4. I. Slutsky *et al.*, *J. Physiol.* **514 (Pt 3)**, 769-82 (1999).
5. J. Minic *et al.*, *Eur. J. Neurosci.* **15**, 439-448 (2002), doi:10.1046/j.0953-816x.2001.01875.x.
6. D. Rochon *et al.*, *J. Neurosci.* **21**, 3819-29 (2001).
7. D. Arbour *et al.*, *J. Neurosci.* **35**, 688-706 (2015), doi:10.1523/jneurosci.1379-14.2015.

Disclosures: A. Mohamed Lafirdeen: None. **E. Tokarski:** None. **P. Ascher:** None. **B. Lamotte d'Incamps:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.04/O8

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant RES220518
NIH Grant RES220483

Title: Scribble in AChR clustering in vertebrate neuromuscular junctions

Authors: *G. XING[1], L. ZHANG[1], Z. DONG[2], H. WANG[3], H. JING[5], W. XIONG[1], L. MEI[4]; [2]Dept. of Neurosciences, [3]Neurosciences, [4]Dept. of Neurosci., [1]Case Western Reserve Univ., Cleveland, OH; [5]Inst. of Life Sci., Nanchang Univ., Nanchang, China

Abstract: The Agrin-Lrp4-MuSK pathway is critical for the neuromuscular junction (NMJ) formation and maturation. However, downstream mechanisms remain poorly understood. To address this question, we attempted to identify proteins whose phosphorylation status is altered by MuSK activity. By using SILAC (stable isotope labeling by amino acids in cell culture) coupled with proteomic analysis, we analyzed MuSK-regulated phosphoproteome and identified Scribble (Scrib), a scaffold protein implicated in cell migration and polarity. We found that Scribble in muscle cells could be serine phosphorylated in response to agrin treatment and was required for agrin-induced AChR clustering. Scrib physically interacted with Lrp4 and rapsyn. Phosphorylation of Scrib promoted the interaction between Scrib and rapsyn/Lrp4, and induced Scrib's self-aggregation. Muscle-specific knockout mice displayed NMJ deficits including reduced AChR cluster size, decreased AChR intensity, diminished junctional folds at adult stage,

suggesting a failure of postsynaptic maturation in Scrib mutant mice. Finally, Scrib protein level was reduced in muscles of 24-months-old mice, suggesting a potential mechanism of NMJ decline in aged mice. Together, these results identified a downstream effector of MuSK to promote AChR clustering and NMJ maturation/maintenance.

Disclosures: G. Xing: None. **L. Zhang:** None. **Z. Dong:** None. **H. Wang:** None. **H. Jing:** None. **W. Xiong:** None. **L. Mei:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.05/O9

Topic: E.09. Motor Neurons and Muscle

Title: Neurochemical and morphological classification of the pontomedullary reticular formation in rhesus macaques

Authors: *R. H. A. JONES[1], S. N. BAKER[2];
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Abstract: Few studies have fully considered the role of the ponto-medullary reticular formation (RF) in movement control, instead focussing on the cerebral cortex and other sub-cortical regions such as the basal ganglia, cerebellum and spinal cord. Previous work from our lab has shown that the RF participates in voluntary limb movements in non-human primates. Additionally, our current knowledge of which neural subtypes are present within the RF nuclei and how these interconnect to form local circuits, and how these circuits receive and process important inputs such as from the cortex, cerebellum or periphery is very limited. This hampers interpretation of the available RF recordings taken from awake animals performing motor tasks. Identifying the neural classes present in the RF and characterising the circuitry is fundamental to our understanding of motor control. Unlike in the cortex, the significance of the organisation of the RF is yet to be elucidated, most likely due to the challenges that come with analysing such non-discrete nuclei. These nuclei, with their lack of clear borders, have been described over the years merely by features such as the size and density of their neurons. A previous study from our group found evidence of 4 distinct neural subtypes within the macaque RF using electrophysiological techniques (Zaaimi, Soteropoulos et al. 2018). Building from this effort to sub-classify RF neurons we hoped to find evidence of and possibly classify these neuron clusters based on their morphology and neurotransmitters.
In this study, we have aimed to comprehensively analyse the neurons of the macaque RF. Looking first at their neurotransmitter profiles, focussing on the inhibitory neurotransmitters GABA and Glycine which have been shown to be involved in reticular transmission, we hope to classify which nuclei have higher expression patterns of which neurotransmitters and how this

differs throughout the length of the brainstem. Combining both immunofluorescence (IF) and fluorescent *in situ* hybridisation (FISH) techniques, we have determined the expression patterns of these neurotransmitters, and using cell counting techniques we are able to quantify these results. Secondly, we assessed the morphology of the cells within these regions of interest using the classical Golgi-Cox neuronal stain. Combining this technique with more general neuronal stains, along with the previously mentioned IF and FISH techniques, we are able to classify the cells by their location, neurochemical profile and also the morphology of their cell body and projections.

Disclosures: R.H.A. Jones: None. **S.N. Baker:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.06/O10

Topic: E.09. Motor Neurons and Muscle

Support: Georgia Gwinnett College SVPASA Seed Grant

Title: Motoneuron synaptic currents in a *Drosophila* seizure mutant

Authors: J. K. VU, D. C. SUGATAPALA, *C. GUNAY;
Sch. of Sci. and Technol., Georgia Gwinnett Col., Lawrenceville, GA

Abstract: Neuronal action potentials are generated by sodium channels, whose mutations underlie some forms of epilepsy. In the fruit fly (*Drosophila*), experimental and computational studies showed a relationship between sodium channels and seizure tendency in the family of seizure-sensitive mutants. In these studies, fly motoneurons were studied in isolation, though their synaptic inputs from premotor interneurons also varied between wildtype and mutant animals. Here, we aim to analyze spontaneous rhythmic current (SRC) inputs to these neurons to quantify their changes. We reproduced statistical analyses of each individual fly's SRC peak widths and heights, as well as the intervals between SRCs, which found substantial differences between the CantonS wildtype and *bang senseless* (bss) mutant fruit flies (Giachello and Baines 2015, *Current Biology*, 25, 2964-2968). This study investigates underlying mechanism(s) of SRC generation that may cause these differences. We hypothesize that each SRC peak is caused by multiple EPSCs (excitatory postsynaptic currents) aggregating on the postsynaptic membrane. We aim to characterize the properties of SRC generation via these underlying EPSCs. We first performed an exploratory qualitative analysis of the data to identify candidate SRCs for further quantitative analysis. For each SRC peak, we counted the number of apparent EPSCs, measured their magnitude, onset time, and time between EPSCs. Distributions of these metrics were compared between wildtype and mutants, and differences in the mean values and variability were

observed. We are searching for recurring patterns in the bss mutant that are distinct from wildtype fruit flies. Understanding these patterns can tell us about changes of circuit anatomy in mutants, including synaptic localization, morphology, strengths, and presynaptic action potential timing. In summary, it will help us understand synaptic contribution to seizure tendency in motor circuits, and allow further investigation of synaptic mechanisms by combining experimental and computational approaches.

Disclosures: **J.K. Vu:** None. **D.C. Sugatapala:** None. **C. Gunay:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.07/O11

Topic: E.09. Motor Neurons and Muscle

Support: Mayo Clinic Office of Research Diversity and Inclusion Research Career Support and Advancement Award

Title: Differences in developmental loss of phrenic motor neurons in an animal model of early onset hypertonia

Authors: *J. E. BRANDENBURG, A. D. BROWN, M. J. FOGARTY, G. C. SIECK; Mayo Clin., Rochester, MN

Abstract: Cerebral Palsy (CP) is the most common motor disability of childhood with hypertonia, specifically spasticity, being the most common sign. Despite CP being poorly predicted by brain imaging, the focus of research has been on the brain being the driver of the motor impairment and hypertonia. By contrast, motor neurons (MNs), via the motor unit and neurotransmitter signaling, are the ultimate target of most clinical therapeutic spasticity treatments in CP, are the final common output of motor control, and are poorly understood in CP. MN development in CP is a critical knowledge gap as the late embryonic and postnatal periods are not only when the supposed brain injury occurs in CP, but is a critical time for spinal cord neuromotor development. Furthermore, while clinical research has focused on improving physical function, respiratory disorders not physical impairment, are among the leading causes of death in individuals with CP. Therefore, using an animal model of early onset hypertonia (*spa* mouse [B6.Cg-*Glrb*^{spa}/J] with a Gly receptor mutation), we have focused our work on evaluating phrenic MN (PhMN) and motor unit physiology. Similar to previous work in other glycine mutants, we hypothesized that *spa* mice will have greater developmental PhMN loss (i.e., less MNs), with PhMNs having a smaller somal size compared to wildtype mice. 12-16 week-old *spa* and wildtype mice from our colony underwent unilateral retrograde labeling of PhMNs via rhodamine phrenic nerve dip. Three days following phrenic nerve dip, mice were euthanized,

perfused with 4% paraformaldehyde, cervical spinal cord excised and processed for longitudinal cryosectioning (70-100 µm) and prepared for confocal imaging. Absolute PhMN counts were obtained using mosaic images of adjacent serial sections, with *spa* mice having 40% fewer PhMNs compared to wildtype mice ($P<0.01$). Somal size measurements (i.e., surface area) were obtained using ImageJ with *spa* mice having a ~30% reduction compared to wildtype mice ($P<0.01$). Developmental PhMN loss and somal size is clearly abnormal in early onset hypertonia and consistent with our hypothesis. We propose that these alterations in developmental PhMN loss may underpin the impaired neuromuscular transmission and force generation in this model of CP, which we are currently exploring.

Disclosures: J.E. Brandenburg: None. **A.D. Brown:** None. **M.J. Fogarty:** None. **G.C. Sieck:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.08/O12

Topic: E.09. Motor Neurons and Muscle

Support: TUBİTAK Grant 116S408
Eskisehir Osmangazi University Scientific Research Projects Commission Grant 2016-1038

Title: Comparisons of different promoters for transgene expression of TAR-DNA binding protein-43 in lower motor neurons of rats

Authors: *E. ULUPINAR[1,2], B. ERCELEN[1], H. A. KAPKAC[3], E. POLAT CORUMLU[2], M. ARSLANYOLU[3];
[1]Anat., [2]Interdisciplinary Neurosci., Eskisehir Osmangazi Univ., Eskisehir, Turkey; [3]Mol. Biol., Eskisehir Tech. Univ., Eskisehir, Turkey

Abstract: Recently, viral vectors have been used for delivery of genes and long-term expression of specific proteins in selected neuron groups. Therefore, viral vector-mediated gene transfer has become an important tool in creating animal models of neurodegenerative diseases. Specificity of promotors plays a critical role in the efficiency of transduction. The aim of this study was to compare the effectiveness of a cell-type specific promoter, Ubiquitin C-Terminal Hydrolase L1 (UCHL1), with a constitutive cytomegalovirus (CMV) promoter in transferring the gene of interest to the motor neurons of rats. Adeno-associated virus (AAV) serotype-9 was used in the design of construction cassette for expression of gene encoding TAR-DNA binding protein-43 (TDP-43) with enhanced green fluorescent protein (eGFP) under the control of two different promoters and inserted between the two inverted terminal repeats. Following hypothermia

anesthesia, the facial vein of 2-day old Sprague-Dawley pups was used for systemic injection of AAV vectors. Animals were sacrificed by intracardiac perfusion under ketamine and xylazine anesthesia on postnatal day 30. Histological sections from the cervical and lumbar spinal cord were used for quantification of lower motor neurons. Ultra-thin sections (80-nm thick) were further processed for ultrastructural analyses by first treating them with 2% osmium tetroxide and then counterstaining with 1% uranyl acetate and 0.2% lead citrate. Our results displayed a significant reduction in the density of lower motor neurons especially in the lumbar spinal cord segments of animals transfected with full-length TDP-43 under the CMV promoter in comparison to those transfected with the UCHL1 promoter. TEM examination of lower motor neurons revealed clustered mitochondria with disorganization in the cristae and the mitochondrial matrix. Various shapes and sizes of vacuoles were observed in the soma as an early stage symptom in the process of neurodegeneration. Collectively, these results indicate that transgene expression of TDP-43 in the newborn rat pups might induce neurodegenerative changes in the lower motor neurons of young adults in a promoter-dependent way.

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Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.09/O13

Topic: E.09. Motor Neurons and Muscle

Title: Comparative toxicities of oximes and oxime scaffolds at the mouse neuromuscular junction

Authors: *K. T. PAGARIGAN, J. B. MACHAMER, N. G. PAPAROIDAMIS, P. M. MCNUTT;
USAMRICD, Aberdeen Proving Ground, MD

Abstract: Exposure to organophosphates (OPs) results in inhibition of acetylcholinesterase (AChE) in both the central and peripheral nervous system, resulting in a cholinergic toxidrome that manifests as seizures and respiratory paralysis at lethal doses. The currently fielded therapeutic regimen for OP poisoning is administration of the muscarinic antagonist atropine in combination with the oxime 2-pyridine aldoxime methylchloride (2-PAM) to reactivate AChE. However, 2-PAM is only modestly effective against a limited range of OP nerve agents and is dose-limited by toxic side effects. Therefore an oxime that protects against a wide range of nerve agents and has lower toxicity is critically needed. MMB4 is a leading candidate for next-generation nerve agent treatment that is more effective at reactivating AChE than 2-PAM. Although MMB4 appears clinically nontoxic at effective concentrations in rodents, MMB4

depresses respiratory function in rabbits due to paralysis of the diaphragm at high concentrations. Recently, we demonstrated that MMB4 antagonizes AChE, pre-synaptic and post-synaptic nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors in a concentration-dependent manner, with IC50 values that are similar to peak plasma levels following *in vivo* administration of toxic doses. In an effort to determine the mechanisms by which various oximes interfere with function of the phrenic neuromuscular junction, we assayed the effects of several oximes and oxime scaffolds on muscle function and neurotransmission via intracellular electrophysiology and vertical tissue bath in mouse diaphragms. The results from this study are expected to inform selection and development of next generation AChE reactivators as well as potentially influence improved clinical development of quaternary ammonium-based non-depolarizing muscle blockers.

Disclosures: K.T. Pagarigan: None. **J.B. Machamer:** None. **N.G. Paparoidamis:** None. **P.M. McNutt:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.10/O14

Topic: E.09. Motor Neurons and Muscle

Support: California Capital Equity LLC
UCLA Bioengineering Fellowship

Title: Optimization for transcutaneous spinal cord stimulation with a multi-electrode array

Authors: *Y. WANG, W. LIU;
Bioengineering, Univ. of California Los Angeles, Los Angeles, CA

Abstract: Transcutaneous spinal cord stimulation (tSCS), an emerging non-invasive neuromodulation, has shown its potential to treat paralysis due to spinal cord injury. Conventional tSCS performs electrical neuromodulation by utilizing one or two large electrodes placed over the back with return electrodes on the belly. One critical issue of the conventional method is the inability to deliver stimulation current to the target location without activating undesired regions. By leveraging the use of a multi-electrode array, we developed a 3D real human model and a unique optimization algorithm that could significantly improve the stimulation focality without performing exhaustive search of the currents delivered from each electrode. The 3D computational human model was derived from cryosection images of the NIH Visible Human Project (VHP), which contains twelve layers including SC, SG, dermis, fat, epidural fat, abdomen, muscle, vertebrae, vertebra disc, CSF, gray matter, and white matter. A computational experiment was conducted to demonstrate the advantages of the multi-electrode

array with our optimization algorithm over the conventional configuration. Target region was designed to be L2~L5 in white matter, which is often the main target region in many clinical applications, such as pain control and lumbosacral spinal network modulation. The simulation results show that conventional configuration produces a voltage Hessian feature of 1500 m/V^2 at the target region and activates non-target segments with a volume of 3.46 mm^3 located in the thoracic level and conus medullaris. The electrode array with our optimization algorithm achieves the same hessian intensity (1500 m/V^2) with a smaller non-target volume (2.21 mm^3) influenced. It achieves selective spinal cord stimulation by maintaining the stimulation intensity but affecting fewer non-target segments.
The computational simulation demonstrates the limitations of conventional tSCS and the feasibility of using a multi-electrode array for selective spinal cord stimulation. The quantitative results show that proposed optimization algorithm can guide the multi-electrode array to maintain the stimulation intensity and affects fewer non-target segments, indicating a great potential for future implementation.

Disclosures: **Y. Wang:** None. **W. Liu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UCLA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents at UCLA and stocks at Niche Biomedical.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.11/O15

Topic: E.09. Motor Neurons and Muscle

Title: Transcallosal functional connectivity between motor cortices in rats probed with unilateral optogenetic stimulation of glutamatergic neurons

Authors: *C. SKOVEN[1], L. TOMASEVIC[1], D. KVITSIANI[2], B. PAKKENBERG[3,4], H. R. SIEBNER[1,5,4], T. B. DYRBY[1,6];
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Abstract: Introduction: Non-invasive Transcranial Brain Stimulation (NTBS) is widely used to induce plasticity in the human brain and bears substantial therapeutical potential. Yet the underlying mechanisms of NTBS are still only rudimentarily understood. In order to delineate

the involvement of different neural components after NTBS of the human primary motor cortex (M1), an optogenetic approach is here carried out, using the rat as a model platform.Many studies do not provide information of their choice of stimulus parameters. We therefore first investigated different input parameters of the optogenetic stimulation and their impact on the electrophysiological (ElPhys) neural response pattern in the contralateral hemisphere.
Method: Young male rats (NTac:SD-M; 4 weeks old) underwent stereotaxic surgery. In right M1, AAV5-ChR2-CaMKIIa was injected and an optic fiber implanted. In left M1 an electrode pair was implanted. Four weeks after surgery animals were exposed to laser light stimulation (447nm) during anesthesia (low isoflurane + dexmedetomidine).The stimulation paradigm consisted of 6 blocks of different and pseudo randomly chosen laser intensities (0.25-10.0mW) with 3*50 trials at different stimulation durations (0.1-10ms), in a randomly intermingled order.
Results: We obtained ElPhys I/O-curves depicting the effect of varying the laser power as well as the stimulation duration on the glutamatergic transcallosal projection neurons.
Longer stimulation durations appear to have a slower return to baseline and seem to introduce late responses 40 ms after stimulus onset. The evoked response peaks around 9 ms after stimulation onset, regardless of stimulation duration.
Discussion: Local neuronal groups are likely differently affected by the different stimulation parameters. The late complex response (after 40ms) might reflect activation of additional local inhibitory/excitatory networks. This simple experiment illustrates the importance of choosing the input parameters for future optogenetic stimulation experiments carefully, in relation to the neurobiological question asked. These observations will be used as starting point for investigating human-like NTBS effects and brain state on top of this model.

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Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.12/O16

Topic: E.09. Motor Neurons and Muscle

Support: NIA/NIH grant AG051442

Title: Troponin I is expressed in α-motor neurons and can act as a potential transcription factor relevant to the age-related neuronal loss

Authors: *K. M. PIEKARZ[1], K. SATARANATARAJAN[2], H. VAN REMMEN[1,2];
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Abstract: Troponin I is a protein commonly associated with cardiac and skeletal muscle tissue, where it forms a complex with troponins C and T and plays a critical role in muscle contraction. Although there is evidence for expression of troponins in non-skeletal muscle cells, including cancer cell lines (troponin T, C and I) and chicken embryonic dorsal root ganglion (troponin C), troponins' function in this non-canonical context still remains to be understood. Here we report for the first time the presence of troponins I and C in α-motor neurons in wildtype C57Bl6J mouse spinal cord using immunoblotting and immunofluorescence staining. RNA-seq analysis revealed a 40-fold increase in troponin I mRNA in spinal cord from old (26-27 months) mice compared to spinal cord from young (9 months) mice. The abundance of troponin I and C proteins is also elevated in aging, 3.77- and 5.16-fold respectively, as measured by western blot in spinal cord lysates from young (6-7 months) and old mice (27-28 months). Importantly, immunofluorescent staining indicates that troponin I translocates to the nucleus in aged but not young α-motor neurons (3-7 months vs 25-29 months), suggesting that it may act as a transcription factor to initiate gene expression changes associated with aging spinal cord. In support of this idea, bioinformatic analysis described in the literature predicts that troponin I contains a DNA-binding domain, and additional reports confirm the ability of troponins to associate with DNA and to regulate it. Thus, we hypothesize that troponin I is a promising candidate for a transcription factor that may regulate age related changes in spinal cord. Ongoing studies using CUT&RUN-seq will define the potential role played by troponin I in aging by identifying the DNA regions troponin I binds to. Furthermore, we will overexpress troponin I in α-motor neurons of young wild-type mice using viral vectors to determine the effect of elevated troponin on α-motor neurons viability and gene expression. Elucidating the role troponin I plays in α-motor neuron aging could allow us to identify the mechanisms that drive age-related α-motor neuron loss, and to develop a strategy aiming at preventing neuronal loss in aging and potentially ameliorate age-related loss of muscle mass and function that results from the loss of muscle innervation.

Disclosures: **K.M. Piekarz:** None. **K. Sataranatarajan:** None. **H. Van Remmen:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.13/O17

Topic: E.09. Motor Neurons and Muscle

Support: NHMRC CJ Martin Fellowship
MNDRIA Project Grant
NHMRC Funding

Title: Size dependent vulnerability of lumbar motor neuron dendritic degeneration in SOD1^{G93A} mice

Authors: *M. J. FOGARTY[1], E. W. MU[2], N. A. LAVIDIS[4], P. G. NOAKES[5], M. C. BELLINGHAM[3];
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Abstract: The motor neuron (MN) soma surface area is correlated with motor unit type. Larger MNs innervate fast fatigue-intermediate (FInt) or fast-fatigueable (FF) muscle fibres in Type FInt and FF motor units respectively. Smaller MNs innervate slow-twitch fatigue-resistant (S) or fast fatigue-resistant (FR) muscle fibres in Type S and FR motor units respectively. In amyotrophic lateral sclerosis (ALS), FInt and FF motor units are more vulnerable, with denervation and MN death occurring for these units before the more resilient S and FR units. Abnormal MN dendritic arbors have been observed in ALS in humans and rodent models. We used a Golgi-Cox impregnation protocol to examine soma size-dependent changes in the dendritic morphology of lumbar MNs in SOD1^{G93A} mice, a model of ALS, at pre-symptomatic, onset and mid-disease stages. In wildtype control mice, the relationship between MN soma surface area and dendritic length or dendritic spine number was highly linear (i.e., increased MN soma size correlated with increased dendritic length and spines). By contrast, in SOD1^{G93A} mice, this linear relationship was lost and dendritic length reduction and spine loss were observed in larger MNs, from pre-symptomatic stages onwards. These changes correlated with the neuromotor symptoms of ALS, and were consistent with neuronal excitability changes observed in patients and in rodent models. At presymptomatic ages, changes were restricted to the larger MNs, likely to comprise vulnerable FInt and FF motor units. Our results suggest that intrinsic or synaptic increases in MN excitability are likely to contribute ALS pathogenesis, not compensate for it.

Disclosures: **M.J. Fogarty:** None. **E.W. Mu:** None. **N.A. Lavidis:** None. **P.G. Noakes:** None. **M.C. Bellingham:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.14/O18

Topic: E.09. Motor Neurons and Muscle

Support: Sigma Xi, Grant in Aid of Research #A-7044998809
Pomona College Neuroscience Department

Title: Membrane-initiated testosterone signaling regulates androgen receptors in a sexually dimorphic motor nucleus

Authors: *L. M. HAETZEL, L. M. RUDOLPH;
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Abstract: The classical theory of steroid hormone signaling suggests that steroid hormones act by altering gene transcription, a process that takes hours to days. However, steroid hormones can trigger molecular signaling cascades within seconds. In recent years, the mechanisms of non-classical steroid signaling have been studied in great detail, including how steroids act at membrane-localized receptors to initiate changes relevant for reproduction and many other behaviors such as learning and memory. The body of work on "non-classical" steroid hormone action has been largely studied for estrogen, and there is far less understanding of where and how non-classical effects of androgens occur. To assess the role of non-classical androgen signaling, we used the spinal nucleus of the bulbocavernosus (SNB), a highly androgen sensitive system involved in rodent reproduction. In this experiment we aimed to determine if testosterone regulates androgen receptor expression in SNB motoneurons via a membrane-initiated mechanism using testosterone conjugated to BSA (T-BSA) which can only act at the membrane. Adult male Long-Evans rats were castrated or left gonadally intact. Five days after castration, animals were treated with T-BSA or vehicle. Following T-BSA injections (500 or 1230 μg, s.c.), animals were euthanized 20 minutes or 2 hours after T-BSA treatment. Immunolabeling for androgen receptors (ARs) was assessed in spinal cord sections containing SNB motoneurons. We found that castration eliminates AR expression in SNB somata. T-BSA fails to rescue the castration-induced decrease in ARs within 20 minutes, but fully restores the percent of AR-positive SNB somata within 2 hours. To our knowledge, these results are the first to demonstrate that testosterone signaling can proceed through a membrane-initiated mechanism in the SNB system. This work suggests the SNB system can be used as a model system for studying rapid androgen action, and contributes to the growing body of literature aimed at understanding the mechanisms of steroid signaling across the neuraxis.

Disclosures: L.M. Haetzel: None. **L.M. Rudolph:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.15/O19

Topic: E.09. Motor Neurons and Muscle

Support: NIH NICHD P2C HD086844-01

Title: Variability and stability of triceps surae M-wave during walking, running, and hopping

Authors: *M. MCLEOD[1], B. POULIOT[2], A. K. THOMPSON[3];
[1]Col. of Charleston, Charleston, SC; [2]Col. of Hlth. Professions, [3]Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: During dynamic cyclical motion, such as walking and jumping, rapid and dynamic changes in lower extremity muscle length and joint motion occur, and likely affect effectiveness of peripheral nerve stimulation and EMG recording. For example, the amplitude of soleus maximum M-wave (M_{max}) is reported to vary across the step cycle during walking (e.g., J Physiol 1999:515:929-939; 2001:530:167-180), suggesting that M_{max}variability be considered in EMG amplitude and reflex size assessment. However, to date, there is no established guideline as to how much stimulation should be given to ensure the achievement of the M_{max} at each part of the step cycle. Thus, to understand the extent and pattern of M_{max} variation over the movement cycle, we are currently examining the triceps surae recruitment curves during walking, running, and two-leg vertical hopping.
In healthy adults without a history of neurological or orthopedic injury, the soleus, medial and lateral gastrocnemius recruitment curves are obtained by stimulating the tibial nerve. In each participant, 1-ms wide single-pulse tibial nerve stimulation is delivered during standing, walking, running, and hopping (at 2Hz pace). The stimulus intensity ranges from the H-reflex threshold to above the M_{max} level. For walking, running, and hopping, the inter-stimulus interval is set to have at least one unstimulated cycle between the stimuli, and several hundred trials are obtained for each task. For walking data, the complete step cycle is divided into 12 bins of equal duration. For running and hopping data, the complete cycle is divided into 8 bins. M-wave recruitment curve is constructed for each bin.
The results to date show that the M_{max} varies within mean±20% range over walking, running, and hopping cycles; the mean M_{max} values across movement cycles are slightly larger than the M_{max} during standing. While the extent of variation is similar across individuals and muscles, the variation pattern over the movement cycle is not consistent across different individuals and muscles. Notably, the stimulus intensity required to achieve the M_{max} markedly differs across the cycle, suggesting that a fixed relative intensity stimulation (e.g., 1.5 x resting M_{max}) would not guarantee the achievement of M_{max} across the entire movement cycle in different individuals and muscles. To confirm these initial observations, further experiments and analyses are currently underway.

Disclosures: M. McLeod: None. **B. Pouliot:** None. **A.K. Thompson:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.16/O20

Topic: E.09. Motor Neurons and Muscle

Support: Villum Kann Rasmussen Fonden
National Institutes of Health (NS069551(AKT)

Title: Effects of stretch velocity on the latency of the human soleus stretch reflex

Authors: *Y. MAKIHARA[1], N. MRACHACZ-KERSTING[2], T. SINKJAER[3], A. K. THOMPSON[4];
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Abstract: When the human soleus muscle is stretched by rapid ankle dorsiflexion, spinal stretch reflex responses are observed in electromyography (EMG). Short Latency Response (SLR, also known as M1) is thought to be mainly Ia afferent origin and Medium Latency Response (MLR, or M2) is presumed to be mainly II afferent mediated. To further understand what are reflected in these responses, in this study, we examined the onset and peak latencies of the M1 and M2 components of human soleus stretch reflex using 5 different ankle rotation velocities. Thirteen adults with no known neurological injuries participated in this study. Participants were seated in a custom-made chair with the knee joint flexed at ≈60° and the right foot fixed onto a servo-controlled electrical actuator, such that the anatomical axis of ankle and the fulcrum of the actuator were closely aligned. To elicit the stretch reflex, 6° of rapid dorsiflexion rotation was applied with randomly varying intervals of 5-7 s, while the participants maintained ≈10% maximum voluntary contraction level of soleus background EMG. Twenty stretches were applied for each of 5 different stretch velocities (74-225 °/s). The onset and peak latencies of M1 and M2 components were determined for each trial and averaged for each condition using a custom made matlab program. The latencies of M1 and M2 were differently affected by different stretch (rotation) velocities: 74±0.4, 144±0.6, 182±1.9, 208±3.0, and 225±4.5 °/s. The onset and peak latencies of M1 changed minimally across the five velocities: 38±0.7 to 38±1.0 ms for onset latency and 44±0.7 to 46±1.1 ms for peak latency. In contrast, the M2 onset and peak latencies progressively shortened with increasing velocities from 64±1.3 to 55±1.4 ms (onset) and from 66±1.3 to 59±1.7ms (peak) ($p<0.05$ by repeated measures ANOVA). The M1 and M2 amplitudes also increased with increasing velocities. The results indicate differing effects of stretch velocity on M1 and M2 onset and peak latencies in the human soleus stretch reflex, likely reflecting different origins of these two components. The minimum difference in M1 latencies across different stretch velocities suggests its sensitivity to dynamic muscle stretch; i.e., Ia afferent origin. The longer M2 latencies with slower stretches are consistent with group II afferent's firing characteristics (i.e., sensitivity to muscle length, which would change with ankle rotation).

Disclosures: Y. Makihara: None. **A.K. Thompson:** None. **N. Mrachacz-Kersting:** None. **T. Sinkjaer:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.17/O21

Topic: E.09. Motor Neurons and Muscle

Support: Villum Kann Rasmussen Fonden
NIH Grant NS069551

Title: Task dependent adaptation and long-term changes in the human soleus stretch reflex

Authors: *N. MRACHACZ-KERSTING[1], U. G. KERSTING[2], T. SINKJAER[3], A. K. THOMPSON[4];
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Abstract: Changing the H-reflex through operant conditioning leads to CNS multi-site plasticity and can affect previously learned skills. In order to further understand the mechanisms of this plasticity, we operantly conditioned the initial (M1) component of the soleus stretch reflex. Unlike the H-reflex, the stretch reflex is affected by fusimotor control, comprises several bursts of activity resulting from temporally dispersed afferent inputs, and may activate spinal motoneurons via several different spinal and supraspinal pathways.
Neurologically normal participants completed six baseline sessions and 24 operant conditioning sessions in which they were encouraged to increase (M1up) or decrease (M1down) M1 size. Five of eight M1up participants significantly increased M1; the final M1 size of those 5 participants was 143±15% (mean±SE) of the baseline value. All eight M1down participants significantly decreased M1; their final M1 size was 62±6% of baseline. Similar to the previous H-reflex conditioning studies, conditioned reflex change consisted of within-session task-dependent adaptation and across-session long-term change. Task-dependent adaptation was evident in conditioning session 1 with M1up and by session 4 with M1down. Long-term change was evident by session 10 with M1up and session 16 with M1down. Task-dependent adaptation was greater with M1up than with the previous H-reflex up-conditioning. This may reflect adaptive changes in the muscle spindle sensitivity, which affects the stretch reflex but not the H-reflex. Because the stretch reflex is directly related to motor function during tasks such as locomotion, M1 conditioning may provide a valuable tool for exploring the functional impact of reflex conditioning and its potential therapeutic applications.

Disclosures: N. Mrachacz-Kersting: None. **U.G. Kersting:** None. **T. Sinkjaer:** None. **A.K. Thompson:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.18/O22

Topic: E.09. Motor Neurons and Muscle

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NIBIB 1P41EB018783 (Wolpaw)

Title: Can combining reflex conditioning and motor practice enhance beneficial plasticity in people with chronic incomplete SCI?

Authors: *A. K. THOMPSON[1], B. A. POULIOT[2], J. R. WOLPAW[4], C. R. GILL[3];
[2]Col. of Hlth. Professions, Hlth. Sci. and Res., [3]Dept. of Hlth. Sci. and Res., [1]Med. Univ. of South Carolina, Charleston, SC; [4]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Center, NY State Dept. of Hlth., Albany, NY

Abstract: People with incomplete spinal cord injury (SCI) often suffer motor disabilities associated with spasticity and poor voluntary muscle control, even after conventional therapy. Operant conditioning that is targeted to an abnormally functioning spinal reflex pathway can initiate wider beneficial plasticity in other spinal and supraspinal pathways, and thereby ameliorates movement disabilities (J Physiol 2018:596: 3469-3491). Indeed, in people with chronic incomplete SCI, down-conditioning of a hyperactive soleus H-reflex can improve locomotion (J Neurosci 2013:33:2365-2375). To optimally integrate reflex conditioning into clinical practice, we are testing a combination of H-reflex conditioning with motor (walking) practice (WP) in people with chronic incomplete SCI; walking practice may accelerate and guide the wider plasticity enabled by reflex conditioning. The combination could be an effective and practical strategy to enhance beneficial plasticity and functional gain. In this study, the reflex conditioning procedure (J Neurosci 2013:33:2365-2375) is immediately followed by WP (3×160 steps or 3×5 min of the person's fastest comfortable walking). The protocol comprises 6 baseline and 30 intervention sessions over 12 weeks (3/wk). In each baseline session, 225 control H-reflexes are elicited while the person maintains natural standing and stable background EMG without feedback on H-reflex size. These trials are immediately followed by WP. In each intervention session, 20 within-session control H-reflexes are elicited first, and then 225 conditioned H-reflexes are elicited while the standing participant is encouraged to decrease H-

reflex size with the aid of visual feedback. WP immediately follows the 225 conditioning trials. For all trials, background EMG activity and M-wave size are kept stable throughout data collection.To date, 4 people with chronic incomplete SCI have completed the combined protocol. All 4 decreased H-reflex size significantly over the course of study; final H-reflex size averaged 52±11(SE)% of baseline. By conditioning session 15, H-reflex size had fallen to 69%, the level that previous studies of H-reflex conditioning alone required 30 sessions to reach; this suggests that the combined protocol may be more efficient and effective. Ten-meter walking speed increased by +70±43% (+0.2±0.09 m/s). Ongoing data collection seeks to confirm these early results and evaluate the persistence of the beneficial effects. Combined protocols of this kind may increase the efficacy of reflex conditioning as a new method that can complement other therapies and enhance recovery after SCI or in other disorders.

Disclosures: A.K. Thompson: None. **B.A. Pouliot:** None. **J.R. Wolpaw:** None. **C.R. Gill:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.19/O23

Topic: E.09. Motor Neurons and Muscle

Title: Operant conditioning of the extensor carpi radialis motor-evoked potential to transcranial magnetic stimulation in people with incomplete spinal cord injury

Authors: *B. A. POULIOT, B. H. S. DELLENBACH, A. K. THOMPSON;
Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC

Abstract: After spinal cord injury, corticospinal excitability and connectivity diminish, resulting in weak voluntary activation of muscles and impaired motor control. Such deficits are at least partially reversible; the excitability of corticospinal pathways increases in association with motor function recovery. Thus, an intervention that improves corticospinal excitability for the affected muscles may enhance motor recovery. Operant conditioning of an EMG evoked potential, which can induce beneficial plasticity in a targeted CNS pathway, may be able to target corticospinal pathways.
Regaining sensorimotor function in the arms and hands is one of the top priorities of individuals with incomplete cervical spinal cord injury (SCI) (J Neurotrauma 2016:33:1958-68. ; J Neurotrauma 2004:21:1371-83. ; J Neurotrauma 2012:29:1548-55.), yet is a major challenge, due to the complex nature of upper extremity motor function (with many degrees of freedom at shoulder, elbow, wrist, and finger/thumb joints). Thus, to enhance upper extremity rehabilitation beyond what conventional therapy alone can achieve, we are currently testing the hypothesis that operant up-conditioning can increase the size of forearm extensor MEP and can improve upper

extremity motor function.
Participants with chronic stable incomplete cervical SCI are exposed to 6 baseline and 24 MEP up-conditioning sessions (3 sessions/wk over 10 wks). In all sessions, wrist extensor, extensor carpi radialis (ECR) MEPs are elicited at 10% above motor threshold while the participant maintains ~30% maximum voluntary contraction level of background EMG activity. In all 225 MEP trials of baseline sessions and the first 20 trials of conditioning sessions, the participant receives no feedback as to MEP size (i.e., control MEPs). Then, in 225 conditioning trials of each conditioning session, the participant is asked to increase MEP size, and receives immediate feedback as to whether MEP was larger than a criterion (i.e., whether the trial was a success). In the first participant with SCI, ECR MEP size increased by 72% over 24 conditioning sessions. The participant also spontaneously reported changes in her hand/arm usage (i.e. open the car door, turn the lights off, press the buttons on the elevator), all of which could not be performed prior to MEP conditioning. This initial case supports our hypothesis that operant up-conditioning can increase ECR MEP size and improve upper extremity motor function in chronic incomplete cervical SCI. Encouraged by the initial results, several more individuals with SCI are currently enrolled into this study.

Disclosures: **B.A. Pouliot:** None. **B.H.S. Dellenbach:** None. **A.K. Thompson:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.20/O24

Topic: E.09. Motor Neurons and Muscle

Support: NIFR1180091

Title: Subtype specific maturation of intrinsic properties drives the orderly recruitment of slow and fast lumbar motoneurons during postnatal development

Authors: *S. A. SHARPLES[1], F. SORRELL[2], G. B. MILES[2];
[1]Univ. of St. Andrews, Fife, United Kingdom; [2]Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Early postnatal development is characterized by considerable changes in fine motor control. A key milestone in the rodent is the emergence of weightbearing locomotion at the end of the first postnatal week, which is associated with increased demands for fine control of hindlimb muscles. Fine motor control is dependent on orderly recruitment of slow and fast motor units with intrinsic properties that match the twitch kinetics of the muscle fibres they innervate. While it is well established that motoneurons undergo diversification of intrinsic properties during this period, we do not understand how maturation of intrinsic properties leads to the

orderly recruitment of fast and slow motoneurons for fine motor control.
To address this, we performed whole-cell patch clamp recordings of motoneurons in lumbar spinal cord slices obtained from mice either shortly after birth (postnatal (P) day 0-4) or following the first postnatal week (P7-12). Passive, action potential and repetitive firing properties were measured from fast and slow motoneurons identified based on delayed or immediate repetitive firing onset during a 5 s current step. Recruitment threshold was defined as the current required to elicit repetitive firing during slow ramp (100 pA/s) current injections. There was no difference in recruitment currents for fast and slow motoneurons at P0-4. However, maximal firing rate was higher in fast motoneurons due to faster action potential kinetics. The orderly recruitment of slow and fast motoneurons was present at older ages due to a reduction in input resistance of fast motoneurons and a depolarisation of the resting membrane potential of slow motoneurons. Interestingly, while higher firing rates were again observed in fast versus slow motoneurons at P7-12, action potential properties did not differ at this age. Instead, maximal firing rates may be higher in fast motoneurons due to changes in the medium afterhyperpolarization, which was found to be shorter in fast compared to slow motoneurons at P7-12. Higher firing rates may be further augmented after the first postnatal week by an increase in persistent inward currents in fast motoneurons only.
Our data suggests that subtype-specific diversification of intrinsic properties leads to the orderly recruitment of slow and fast motoneurons and may provide greater fine motor control of the hindlimbs following the emergence of weightbearing locomotion.

Disclosures: **S.A. Sharples:** None. **F. Sorrell:** None. **G.B. Miles:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.21/O25

Topic: E.09. Motor Neurons and Muscle

Title: Post discharge activity of spinal motor neurons across postnatal development in mice

Authors: *F. L. SORRELL[1], S. A. SHARPLES[2], K. T. SILLAR[3], G. B. MILES[3];
[1]Univ. of St Andrews, St Andrews, United Kingdom; [2]Neurosci., Univ. of Calgary, Calgary, AB, Canada; [3]Univ. St Andrews, St Andrews, United Kingdom

Abstract: In spinal motor neurons of the neonatal mouse and *Xenopus laevis* larvae (Picton et al, 2017), changes in post-discharge excitability can be caused by hyperpolarisation or depolarisation of a neuron following a period of intense repetitive firing. These activity-dependent changes are known to produce cumulative effects on the output of rhythmically active motor networks, thus changing subsequent locomotor behaviour. For example, recent work has shown that the ultra-slow afterhyperpolarisation (usAHP) facilitates a short-term motor memory

trace within these networks, providing a fatigue-prevention mechanism. While this work has highlighted the importance of understanding the contribution of post-discharge activity to motor network output, the full diversity and development of these activity-dependent phenomena remains undetermined. We therefore characterised the post-discharge activity of spinal motor neurons across a critical developmental period surrounding the emergence of weight-bearing locomotion. Whole-cell patch-clamp recordings were obtained from lumbar motor neurons (n=166) in spinal slice preparations from neonatal mice at postnatal days two to eleven (P2-P11). We found a change in the ratio of afterdepolarisations (ADPs) to afterhyperpolarisations (AHPs) between pre-weight bearing (P1-P6) and weight bearing stages (P7-P11). Pre-weight bearing animals show a larger ratio of ADPs to AHPs (70:30, n=101), with the converse being true in weight bearing animals (40:60, n=65). During the emergence of weight-bearing (P7-P8), a particular increase in the occurrence of ultra-slow afterhyperpolarisations was observed. Interestingly, individual neurons were found to be capable of producing a range of post-discharge activities, as revealed by the reversal of ADPs and strengthening of AHPs (n=15) upon blockade of calcium conductances (cadmium chloride, 100μM). Furthermore, we reveal that the post-discharge activity of motor neurons is adaptable, as evidenced by modulation of ADPs and AHPs via application of 5-HT (n=11) -- a key neuromodulator during early postnatal development of spinal motor networks. In summary, these data highlight changes in the expression profile of diverse and adaptable post-discharge activities in spinal motor neurons across an important window of postnatal development. These findings advance our understanding of the roles that specific neuronal properties play in determining motor network output and may have implications for studies of neuropathologies affecting movement.
Reference: Picton *et al.* 2017, *J Neurophysiol.* 118: 1070

Disclosures: **F.L. Sorrell:** None. **S.A. Sharples:** None. **K.T. Sillar:** None. **G.B. Miles:** None.

Poster

066. Motor Neuron I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 066.22/O26

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant NS104436

Title: Preterm fetal hypoxia-ischemia increases abnormal motor activity in the neonatal rabbit

Authors: *C. F. CAVARSAN, P. STEELE, M. WESTEFELD, K. A. QUINLAN;
Biomed. and Pharmaceut. Sci., Univ. of Rhode Island, Kingston, RI

Abstract: Cerebral palsy (CP) has a prevalence of ~1 in every 400 live births, and it is caused by a variety of factors related to early brain damage, resulting in permanently impaired motor

control, marked by muscle stiffness and spasticity. To study mechanisms of motor dysfunction, we use the rabbit hypoxia-ischemia (HI) model of developmental injury at 79% pregnancy (E25). Previous characterization of the phenotypes (which vary from severe to unaffected) was based on a modified Ashworth score. The aim of this study was to add more detailed behavior analysis and correlate behavior to injury. During the neonatal period (P1-P5) motor function was analyzed in HI-injured (n=27) and sham (n=28) kittens. Qualitative scored data from previously described behavioral scoring showed that HI-injured animals could not maintain their prone position and showed a delayed righting reflex compared to sham animals. Surprisingly, using more detailed behavioral analysis (AnyMaze Video Tracking) in open field tests showed that unaffected and mildly affected kits spend more time moving, travel greater distances and at a higher speed, compared to sham animals. Movement in a straight line forward (instead of circular) showed higher mean score in unaffected and mild HI animals, confirmed by the tracking analysis. Our finding suggests that mild and "unaffected" kits after prenatal HI may show a subtle phenotype that includes increased locomotion and hyperactivity. These results are preliminary and will be compared to extent of injuries to the brain in follow up studies.

Disclosures: C.F. Cavarsan: None. **P. Steele:** None. **M. Westefeld:** None. **K.A. Quinlan:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.01/O27

Topic: F.01. Neuroethology

Support: NSF #1743475

Title: Worm-like turning: Planning coordination of long soft body movements

Authors: P. PANDIT[1], A. KANDHARI[2], Y. WANG[1], ***K. A. DALTORIO**[1];
[2]Mechanical and Aerospace Engin., [1]Case Western Reserve Univ., Cleveland, OH

Abstract: Earthworms locomote using traveling waves of segment contraction and expansion. Due to hydrostatic coupling, an increase in segment length causes a decrease in segment diameter. Segments can also bend. The mechanics of the body result in a large range of body shapes that both comply with the environment and contribute to directed locomotion. Path planning in soft robotics is difficult due to the large number of imprecise degrees of freedom, the constraints from each segment's interaction with the environment, and the large state space. This research focuses on the formation of a path for a soft earthworm-like robot to travel from an initial to a desired position and configuration, without any of the segments undergoing slip. The locomotion mechanics of the robot is simulated in MATLAB, where we explore path planning

algorithms based on Rapidly-exploring Random Trees (RRT) and curve generation algorithms that form a path by taking robot's constraints into consideration. Our work shows the challenges of turning a long segmented robot to return to a specific location. Better understanding of how animals and robots can plan and execute paths will improve design and control of future robots with application in infrastructure maintenance, medicine and exploration.

Disclosures: P. Pandit: None. **A. Kandhari:** None. **Y. Wang:** None. **K.A. Daltorio:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.02/O28

Topic: F.01. Neuroethology

Support: NSF #1743475

Title: Structural skin for soft body locomotion: Worm robots that eliminate hard joints

Authors: *A. KANDHARI[1], A. MEHRINGER[1], D. JOLLEY[1], H. J. CHIEL[2], R. D. QUINN[1], K. A. DALTORIO[1];
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Abstract: Soft-bodied animals, such as earthworms or sea slugs, use the surface of their body to crawl or swim. Implementing this type of locomotion on a robot can have many applications such as search and rescue, inspection of pipes and medical procedures. Our group in particular has been approximating worm-like locomotion with robots composed of soft helical tubing connected in a mesh of pinned rhombuses. The mesh provides an inverse relationship between segment length and diameter, akin to the volumetric constraints of muscular hydrostats. The addition of a smooth skin can separate the inside and outside of the body to protect the robot from debris and catching on rough terrain. The skin's structural properties will be critical to performance. Such a skin requires high strain, low stiffness, and long fatigue life. Here we present two new robots: FabricWorm and MiniFabricWorm. We explored the application of fabric in soft robotics and how textile can be integrated along with other structural elements, such as three-dimensional printed parts, linear springs, and flexible nylon tubes. Because of this fabric skin, the mesh structure of FabricWorm requires only one third of the number of rigid pieces as compared to its predecessor Compliant Modular Mesh Worm-Steering (CMMWorm-S). Furthermore, the structure of MiniFabricWorm consists of no rigid components. Thus, the addition of the skin simplifies the structure. This poster presents the design of such a mesh and its limitations in terms of structural softness. We experimentally measured the stiffness properties of these robots and compared them directly to their predecessors. FabricWorm and

MiniFabricWorm are capable of peristaltic locomotion with a maximum speed of 33 cm/min (0.49 body-lengths/min) and 13.8 cm/min (0.25 body-lengths/min), respectively. Fabric-integrated mesh is light, highly flexible and cheap to manufacture when compared to its non-fabric counterparts. These characterizations will help us with future work in which structural skins can be integrated with even softer materials such as molded polymers, novel actuators, or soft 3D printed parts.

Disclosures: **A. Kandhari:** None. **A. Mehringer:** None. **D. Jolley:** None. **H.J. Chiel:** None. **R.D. Quinn:** None. **K.A. Daltorio:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.03/O29

Topic: F.01. Neuroethology

Support: National Institute of Neurological Disorders and Stroke (R01NS094403)
JSPS Research Fellowships for Young Scientists 18J00526
JSPS KAKENHI JP19K16191

Title: Whole ganglion mapping for various mechanosensation in the leech nervous system using voltage-sensitive dye imaging

Authors: *Y. TOMINA[1,3], K. OKA[4], D. A. WAGENAAR[2];
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Abstract: Animals discriminate different intensities of mechanical stimulation to their body surface, e.g. light touch, pressure, and noxious stimuli. Neuronal representation of such somatosensory perception by neuronal population in the large-scale networks is largely unknown especially at the level of single cell membrane potentials. Medicinal leeches have been adopted as one of the model animals for understanding neuronal network mechanisms underlying mechanosensation from sensory input to motor outflow. Electrophysiological studies have identified interneurons monosynaptically connecting with pressure neurons and motor neurons (Lockery and Kristan, 1990). In addition, recent studies using voltage-sensitive dye (VSD) imaging demonstrated that more interneurons were recruited in response to intracellular pressure cell stimulation (Tomina and Wagenaar, 2017) or tactile stimulation (Fathiazar et al., 2018). Here, we aim to complete comprehensive canonical mapping of the leech ganglion for understanding multiple mechanosensory representations by neuronal population activities in the leech, *Hirudo verbana*. We focused on nine types of mechanosensation as the target of intracellular stimulation: light touch (Tv, Td, and Tl cells), pressure (Pv and Pd cells),

nociception (N1 and N2 cells), light touch+pressure (Tv+Pv and Td+Pd cells). Using a double-sided microscope that we previously developed (Tomina and Wagenaar, 2017), we applied VSD imaging to detect the changes of the graded membrane potential of neuronal cell bodies on both ventral and dorsal side of the ganglion, simultaneously. Based on the canonical maps according to coherence information of stimulation frequency of the particular type of mechanosensory neuron in isolated segmental ganglia (collected from 10-20 animals for each type of stimulation), we described which neurons were consistently recruited and how many degrees they responded to a particular stimulus. Our major findings are as follows: (1) Neurons involved in light touch sensation were widely overlapped with those involved in pressure sensation, (2) In comparison to the case of stimulation of light touch cells, many more neurons strongly and consistently responded to pressure cell stimulation, (3) Combination of light touch and pressure stimuli, mimicking natural tactile stimulation, more strongly depolarized those neurons, (4) Stimulation of nociceptive neurons recruited fewer neurons than in the case of the pressure neuron stimulation, while nociceptive stimulation elicits strong motor responses.

Disclosures: **Y. Tomina:** None. **K. Oka:** None. **D.A. Wagenaar:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.04/O30

Topic: F.01. Neuroethology

Support: NIH grant NS094403

Title: Form and function: Connectivity and activity maps of behavioral circuits in the medicinal leech

Authors: M. M. ASHABER[1], Y. TOMINA[1], E. A. BUSHONG[2], K. Y. KIM[2], T. DEERINCK[2], M. H. ELLISMAN[2], ***D. A. WAGENAAR**[1];
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Abstract: Most people would agree that behaviors in invertebrate as well as vertebrate animals occur as the result of a tight interplay between multiple neurons, enabled by highly specific neuronal connectivity. However, the relationship between activity (function) and connectivity (form) in most neuronal circuits remains poorly understood. To bridge this gap, combining functional and anatomical imaging is needed, which is exactly what we are now doing in the medicinal leech.
The medicinal leech (Hirudo verbana) is an ideal model for voltage-sensitive dye (VSD) imaging as it almost literally wears its soul on its sleeve: all the 400 or so neurons of its segmental ganglia are located in a single shell around a central neuropil. This allowed us to record from nearly all

of them in parallel using just two image planes. With this setup, we recorded neuronal activity during (fictive) swimming and other behaviors, before embedding the functionally imaged ganglion in a resin in preparation for electron microscopy.
We then obtained an anatomical image stack from that same ganglion using serial block-face electron microscopy (SBEM). This resulted in a 22 teravoxel rendering of the 600 x 600 x 200 um ganglion; sufficient detail to trace the majority of neuronal arbors. Importantly, an intermediate stage of X-ray imaging allowed us to map the physiological image stack onto the anatomical image stack. Thus we were able to identify the cell bodies from which we recorded with VSDs in the SBEM data.
As a first example of a behavioral circuit, we fully reconstructed one motor neuron (an excitor of dorsal longitudinal muscles known as DE-3) and identified its presynaptic partner cells by tracing back from all of its input synapses to the corresponding somata. As a proof of principle, we determined that a high synapse count for a given presynaptic partner correlates with a tighter functional relationship between that neuron and DE-3 during swimming. We also verified the anatomical presence of many synaptic connections that had previously been described in the physiological literature.
Since DE-3 is a common output of the pattern generators for several behaviors that are included in our VSD recordings, our data set promises new insight into how nervous systems are able to produce diverging behavioral outputs using a single common circuit.

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Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.05/O31

Topic: F.01. Neuroethology

Title: Neural correlates of adaptive responses to changing load in feeding *Aplysia*

Authors: *J. P. GILL[1], H. J. CHIEL[2];
[1]Biol., [2]Biology, Neurosciences, Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Animals flexibly and robustly respond to changing environmental conditions. In feeding, animals encounter foods of varied toughness, shape, and texture. Ingesting these foods requires adjusting motor signals to the body to compensate for this variability. Even as the animal swallows food, the mechanical properties of the food begin to change. Thus, the problem to solve changes as an animal solves it. Understanding this dynamic reorganization of motor control in animals could help us build better engineered devices, such as autonomous robots, or more flexible prosthetic devices for humans.

The marine mollusk *Aplysia* shows a complex adaptive response to mechanical loading during feeding (Hurwitz and Susswein 1992). How do *Aplysia* adapt feeding motor programs to changing load? We sought to fully characterize this behavior and its neural control.
When feeding on natural seaweed, which can be highly branched and is composed of tough stipes and delicate leafy blades, *Aplysia* show high variability in the duration and intensity of forces generated as they ingest food. To understand the neural mechanisms of this ability to adjust motor programs, we developed a set of simplified, uniform food stimuli to help us parse the variability seen in natural food: uniform thin seaweed strips, thick strips, and tear-resistant strips. At the same time, we used a technique for implanting electrodes in intact behaving animals on a key muscle and key nerves important for feeding behaviors so that we could record motor programs during feeding (Cullins and Chiel 2010). We measured force during swallowing, and we focused on the activity of motor neurons responsible for generating retraction force, the power stroke of swallowing, which have axons that project onto buccal nerve 2 (BN2): identified neurons B3, B6, and B9 (Lu et al. 2015).
As expected, the responses to natural food were highly variable in both duration and overall activity (mean rectified voltage) on BN2 during the retraction phase. In contrast, when feeding on uniform foods, the variability was much lower. For uniform thin strips, both the duration and mean activity tended to be low; for thick strips, the duration and mean activity tended to be higher; for tear-resistant strips, the duration and mean activity tended to be intermediate. All these responses to uniform food fell within the broad region observed as animals fed on natural food.
The results support the hypothesis that animals may dynamically assemble the necessary motor outputs in response to ongoing changes in the environment, some of which are due to the animal's own behavior. This may be a general principle for the function of motor systems in other animals and in humans.

Disclosures: J.P. Gill: None. **H.J. Chiel:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.06/O32

Topic: F.01. Neuroethology

Title: Investigating signaling direction of interneurons B4/B5 in *Aplysia californica*

Authors: *Y. HUAN[1], J. P. GILL[1], R. K. SMOLDT[2], H. J. CHIEL[3];
[1]Biol., [3]Biology, Neurosciences, Biomed. Engin., [2]Case Western Reserve Univ., Cleveland, OH

Abstract: As interneurons within the neural circuitry that controls feeding behavior in *Aplysia*, B4/B5 modulate the activity of other motor neurons. Their inhibitory effect on several motor

neurons plays an important role in coordinating rejection behavior, so investigating the control of activity in the B4/B5 neurons will contribute to an understanding of the neural circuitry controlling feeding. In previous experiments, we observed that action potentials from the periphery sometimes preceded somatic signals in B4/B5. Generally, sensory neurons signal from the periphery to the central nervous system. We therefore hypothesized that, in addition to its interneuronal role, the axon of B4/B5 may have a sensory function. To test the hypothesis, we examined the signaling direction of B4/B5 both *in vitro* and *in vivo*. In *in vitro* experiments, we isolated the buccal ganglia and the buccal mass, and intracellularly recorded from both B4 and B5, and extracellularly recorded their axons on buccal nerve 3 (BN3) at two locations. In *in vivo* experiments, we implanted several electrodes in the living animal, including two on BN3. With only BN3 attached to the feeding apparatus *in vitro*, our data showed that touching the muscle of the feeding apparatus generated action potentials in the periphery, and then activated the B4/B5 soma. The action potentials evoked by the touch were still observed when the buccal ganglia were in a high divalent cation (Hi-Di) solution, suggesting the activation of B4/B5 was not caused by polysynaptic inputs. We demonstrated that magnesium chloride that anesthetized the feeding apparatus blocked the generation of action potentials at the periphery, whereas a high cobalt, low calcium solution did not block sensory signals, supporting the hypothesis that B4/B5 axons have a sensory role. In both *in vitro* and *in vivo* experiments, we observed signals propagating from the central nervous system to the periphery during a rejection motor pattern, while peripheral signals propagated in the other direction. These results may be important for understanding the function of B4/B5 during feeding behavior both as interneurons and sensory neurons.

Disclosures: Y. Huan: None. **J.P. Gill:** None. **R.K. Smoldt:** None. **H.J. Chiel:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.07/O33

Topic: F.01. Neuroethology

Support: BRAIN Grant U01NS10837

Title: Large-scale voltage-sensitive dye recording of neuronal activity in the brain of berghia, a newly introduced laboratory species

Authors: E. S. HILL, *W. N. FROST;
Ctr. for Brain Function and Repair, Rosalind Franklin Univ., North Chicago, IL

Abstract: Gastropod mollusks are valuable for elucidating neural mechanisms of behavior. The present study is part of a new 5-PI NIH BRAIN Initiative project (P. Katz, V. Lyzinski, W. Frost,

J. Lichtman and D. Lyons) to establish the nudibranch mollusk *Berghia stephanieae* as an experimentally tractable system for large-scale connectomics research. *Berghia's* advantages include: a short generation time, being easy and inexpensive to obtain and maintain in the laboratory, and having a nervous system complexity between that of *C. elegans* and *Drosophila*. This project component is focused on large scale optical recordings of *Berghia's* neurons - a challenge given its smaller brain and neuronal somata compared to those of the more widely studied marine mollusks we have previously worked with (*Tritonia* and *Aplysia*). Here we report progress using the fast voltage-sensitive absorbance dyes RH155 and RH482 and a 464 element photodiode array sampled at 1,600 Hz, an approach well-suited to recording the action potentials of several dozen neurons simultaneously. Recordings of spontaneous neural activity in the dorsal cerebropleural ganglia of isolated brain preparations revealed that many neurons were active at rest. Some of these were tonically active, whereas others fired in series of short bursts over the time course of the 2 - 10 min recordings. Raw data sets were spike-sorted using independent component analysis (ICA) to obtain action potential recordings from 30 - 55 individual neurons. The exact XY locations of all recorded neurons were displayed on an aligned photograph of the brain, computed from the two dimensional Gaussian distributions of signal strength drawn from the ICA weights matrix. A consensus community detection clustering routine was used to determine the functional ensembles present in each recording, which were then visualized as network graphs. *Berghia*'s lensed eyes are located adjacent to the brain. Light applied to eyes kept in the dark during the optical recordings produced phasic alterations of firing in multiple neurons. From this work we have demonstrated that *Berghia* is amenable to large-scale imaging of neuronal activity with single neuron precision, and thus has promise for high throughput analysis of the neural basis of behavior.

Disclosures: E.S. Hill: None. **W.N. Frost:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: F.01. Neuroethology

Support: NIH BRAIN U01-NS108637

Title: Spatial vision from a low-resolution eye

Authors: *P. D. QUINLAN[1,2], K. E. FISCHER[3], B. DRESCHER[1,2], J. W. LICHTMAN[4], P. S. KATZ[1,2];
[1]Biol., [2]Neurosci. and Behavior, [3]Biochem. and Mol. Biol., Univ. of Massachusetts Amherst, Amherst, MA; [4]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: There is a growing realization that low-resolution eyes can be used for complex visual tasks. It was previously thought that the simple eyes of nudibranch molluscs, comprised of a spherical lens, pigment cells, and about five photoreceptor cells, were not capable of providing spatial information about the environment. We examined the ultrastructure of the eye in the nudibranch, *Berghia stephanieae*. Thin sections were acquired using automated tape-collecting ultramicrotome and electron microscopy (EM). We identified a previously unreported conical structure in the back of the eye, possibly of rhabdomeric origin, that may function in photoreception. We are further examining the connectivity of photoreceptor cells in the eye using both light level and serial EM reconstruction. Despite the simplicity of the eye, we found behavioral evidence of spatial vision in *Berghia*; when placed in the center of a circular tank surrounded by a white wall interrupted with a single black stripe, *Berghia* reliably navigated toward this stripe and crawled up the wall of the tank. The presence of a visual target elicited directed locomotion; in the absence of a visual target, locomotor paths were undirected, longer, and more convoluted. *Berghia* responded to stripes as thin as 15° of visual angle, but seemed to prefer 45°. We hypothesized that *Berghia* were attracted to the stripe because they seek shelter under objects. *Berghia* did not crawl towards visual targets that lacked features of an accessible shelter, such as a white stripe or a black stripe that did not reach the floor of the arena. Navigation toward black stripes was reduced in hungry animals, a motivational state that typically increases exploratory behaviors such as foraging. These results suggest the simple eyes of *Berghia* provide it with spatial information that it uses to navigate toward targets that appear to be shelters. We aim to determine the neural mechanisms that allow a structurally simple eye to guide spatial vision used for motivated behaviors.

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Poster

067. Invertebrate Sensory-Motor Integration

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.09/O35

Topic: F.01. Neuroethology

Support: IMS PBRF to C. Radford and J. Montgomery
University of Auckland Doctoral Scholarship to A.S.Flood
IMS PBRF to M. Goeritz and C. Radford

Title: Sound production in decapod crustaceans - Behavioral contexts and a newly found role for the circuits of the stomatogastric nervous system

Authors: *M.-L. GOERITZ[1], A. S. FLOOD[2], C. RADFORD[2];
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Abstract: We show that decapod crustaceans are able to produce a variety of sounds. These sounds are associated with a wide range of different behavioral contexts. We show a group of sounds that are specifically related to male competition during mating behavior. Another set of sounds, found in both sexes, coincides with excitation and anticipation of feeding. Interestingly, only a subset of the observed sounds is produced by well-described mechanisms such as leg/claw stridulation. Other sounds are internally produced, without any observable movement of appendices. By simultaneously recording muscle or nerve activity in freely behaving crabs, we show strong evidence that at least one of the underlying mechanisms for these sounds is the movement of the gastric teeth inside the crustacean stomach. This points towards a new and exciting role for the circuits of the stomatogastric nervous system. The need to control stomach teeth movement, not only in the presence of food, but in a variety of different behavioral contexts, might explain the puzzlingly complex array of neuromodulation and sensory feedback that has been found in the stomatogastric nervous system.

Disclosures: M. Goeritz: None. **A.S. Flood:** None. **C. Radford:** None.

Poster

067. Invertebrate Sensory-Motor Integration

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Title: Sensory encoding of 'natural' forces during walking: Effects of applying joint torques from single steps of freely moving insects

Authors: *S. N. ZILL[1], C. J. DALLMANN[2,3], C. M. HARRIS[1], J. SCHMITZ[3], A. BUSCHGES[4];
[1]Biomed. Sci., Joan C. Edwards Sch. of Medicine, Marshall Univ., Huntington, WV; [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [3]Biol. Cybernetics, Bielefeld Univ., Bielefeld, Germany; [4]Biocenter Cologne, Univ. of Cologne, Koeln, Germany

Abstract: Sense organs that detect forces in legs can aid in control of walking by adapting activities of individual muscles and synergists to variations in load. In insects, forces on the legs generate cuticular strains that are monitored by campaniform sensilla. Individual receptors can signal force increases or decreases depending upon their location and orientation. Previous studies in stick insects and cockroaches showed that forces applied to the legs using linear ramp-

and-hold stimuli could activate synergist muscles that generate substrate adhesion and support, but substantial adaptation could occur to sustained stimuli. Adaptation was greatly reduced when forces were applied using more 'natural' non-linear stimuli whose time profiles and magnitudes were based on joint torques calculated in freely walking animals. However, those studies only examined the mean values of torques, while torque profiles could show substantial variations in individual steps. To test the effects of variability in forces, torque waveforms of individual steps of freely moving stick insects were selected that had the lowest and highest average values (from experiments in 5 animals). Initial experiments have examined whether variations in forces are encoded by the sensory receptors (tibial campaniform sensilla) in cockroaches and stick insects. In tests applied at low force levels, receptors that encode increasing forces (cockroach proximal tibial sensilla, stick insect Gp 6B) show continuous discharge with little adaptation during the initial sustained rising phase of force. Discharges closely reflect variations in force dynamics (dF/dt) but also show considerable hysteresis to transient force decreases. These decrements do not elicit firing in receptors that encode force decreases (cockroach distal tibial sensilla, stick insect Gp 6A) but effectively modulate firing of receptors that encode force increases. Receptors that encode force decreases only consistently discharge near the end of force application when forces decline toward zero. Similar characteristics are also found in sensory discharges to higher levels of force but dynamic sensitivity is reduced when changes are imposed upon tonic firing that reflects the force level. Preliminary experiments indicate that similar effects of force variations occur in activation of a synergist muscle (tibial flexor). Our findings are consistent with the ideas that phasic and static characteristics do not function as separate components in 'natural' behaviors and that dynamic sensitivities play a major role in force sensing and in the generation of motor behavior.

Disclosures: **S.N. Zill:** None. **C.J. Dallmann:** None. **C.M. Harris:** None. **J. Schmitz:** None. **A. Buschges:** None.

Poster

067. Invertebrate Sensory-Motor Integration

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Title: Sensorimotor processing in freely-moving Hydra vulgaris

Authors: *W. YAMAMOTO, R. YUSTE;
Columbia Univ., New York, NY

Abstract: Cnidarians have a simple nervous system called a nerve net. A nerve net is interconnected neurons diffused across the body, and it lacks ganglia or any form of cephalization. With this simple nervous system, cnidarian animals sense outside world, integrate the information, and generate selective behaviors. Yet, how a simple nerve net can encode these sensorimotor mechanisms and behaviors remains unknown. To address this, we use a cnidarian, *Hydra vulgaris*. *Hydra* is a small (0.5-1.5 cm in length) fresh-water polyp, with only about 600-2000 neurons. We previously generated transgenic animals expressing the genetically-encoded calcium sensor GCaMP6s in the entire nervous system. Being transparent, *Hydra* provides a unique opportunity to image the entire neural activity of a freely-moving animal under a microscope. Indeed, using mounted *Hydra's*, we previously identified three non-overlapping neural circuits that are each associated with a specific motor behavior: contraction burst (CB) neurons associated with longitudinal contractions, rhythmic potential 1 (RP1) neurons associated with elongations, and rhythmic potential 2 (RP2) neurons associated with radial contractions. When one of these neural circuits is active, the others appear suppressed. This suggests that neural circuits regulate one another to conduct a specific behavior in an exclusive fashion. To test how the spatiotemporal dynamics of CB and RP1 activity regulate body contractions and elongations in freely moving animals, which are constantly influenced by sensory input, we have now established a computational method to extract spikes of CB and RP1 from calcium imaging movies of freely moving *Hydra*. We find two types of contractions: 1) RP1 frequency-independent contractions and 2) RP1 frequency-dependent contractions. The RP1 frequency-independent contractions appear to be caused by the sensory input from tentacles. On the other hand, RP1 frequency-dependent contractions occur as result of changes in internal neural dynamics, by which RP1 frequency gradually reaches to 0.5 Hz, while the animal expresses a sophisticated behavior called "somersaulting". In future experiments, the causal relationship between neural activity and behavior will be tested using optogenetics. The results from this study, using a basal metazoan, may provide basic insights of how neural circuits encode behavior.

Disclosures: W. Yamamoto: None. **R. Yuste:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.12/O38

Topic: F.01. Neuroethology

Support: NSF EDGE

Title: Behavioral and neural responses to thermal stimulation in Hydra vulgaris

Authors: C. N. TZOUANAS[1], *S. K. KIM[2], K. N. BADHIWALA[1], J. T. ROBINSON[2,1,3]; [1]Bioengineering, [2]Electrical and Computer Engin., Rice Univ., Houston, TX; [3]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: As a model organism for studying the relationship between neural activity and behavior, Hydra vulgaris offers several opportunities. The small size and transparent body facilitates fluorescent imaging of the entire nervous system with single-cell resolution. Additionally, the number of neurons in the animal can change by more than a factor of five without a clear change in animal behavior. These qualities raise opportunities to discover how behaviors remain stable despite changes to the number and connectivity of neurons in a circuit. To make use of these properties, it is critical to establish quantitative relationships between stimuli, behavior, and neural activity. Here, we show the first quantitative measurements of Hydra's response to thermal stimulation and the corresponding activity of the nervous system. We find that when exposed to elevated temperatures, Hydra contracts and the magnitude of this contraction (measured as a change in body volume) depends on the magnitude of the thermal stimulus. These contractions are accompanied by synchronous oscillatory activity of neurons in the animal's peduncle, which shows a temperature-dependent oscillation frequency. Overall, *Hydra*'s response to thermal stimulation provides an experimental paradigm to better understand simple sensorimotor transformations and neural circuits in a model organism with a highly dynamic neural architecture.

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Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

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Program #/Poster #: 067.13/O39

Topic: F.01. Neuroethology

Support: NSF EDGE

Title: Sensory-motor behaviors in hydra captured with high-throughput automated tracking technology

Authors: *K. N. BADHIWALA[1], B. AVANTS[2], J. T. ROBINSON[3];
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Abstract: The cnidarian *Hydra Vulgaris* has unique properties as a model organism for neuroscience. Its transparent millimeter-sized body facilitates whole-nervous system imaging in microfluidic devices. Additionally, each cell in the nervous system is replaced every 20 days and the number of neurons (as well as their connectivity) changes based on nutrient availability. Given this highly dynamic nervous system, it is possible to study how neural circuits rewire to maintain stable behaviors. However, we must first understand the animal's behavioral repertoire. While prior work spanning over 300 years have reported a variety of behavioral responses to optical and thermal stimuli, there has been no large-population studies of the animal's sensory-motor responses or their kinetics. The primary difficulty in performing these types of quantitative sensory-motor studies is the relatively slow movements of Hydra. Typical locomotion velocities range from 1-100 mm/day. To overcome this challenge, we developed a high-throughput Hydra Imaging Platform for Science, Technology, and Engineering Research (HIPSTER) that combines microfluidics, optical and thermal stimulation, time-lapse imaging, and automated animal tracking. With this platform, we were able to quantify Hydra's photo-aversive behavior as well as photo- and thermo-taxis. Critically, we were able to show how these behaviors depend on the animal's prior culture conditions. These well-defined sensory-motor behaviors and high-throughput methods to assess them open up the ability to study how neural circuits organize and reorganize to maintain specific animal behaviors.

Disclosures: K.N. Badhiwala: None. **B. Avants:** None. **J.T. Robinson:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.14/O40

Topic: F.01. Neuroethology

Support: The Grass Foundation
Denison University Helen L. Yeakel Summer Research Fund

Title: Electrophysiological and motor responses to chemosensory stimuli in isolated cephalopod arms

Authors: *H. J. RHODES[1,2], K. E. FOUKE[1,2];
[1]Biol. and Neurosci., Denison Univ., Granville, OH; [2]Grass Lab., Marine Biol. Lab., Woods Hole, MA

Abstract: Behavioral and anatomic studies have provided strong evidence that octopuses and other cephalopods can detect chemical stimuli with receptors on their arms and suckers. However, the physiology of the chemosensory systems in cephalopod arms and suckers has not been explored. We attempted to record neural activity in the brachial nerves of adult, wild caught *Doryteuthis pealeii* (n=17 individuals) and juvenile, captive-raised *Octopus bimaculoides* (9 individuals, 21 arms) while arms were exposed to environmentally relevant chemical stimuli. Individual arms were surgically removed from euthanized squid or anesthetized octopuses and a suction electrode was attached to the nerve cord; arms were perfused with oxygenated, filtered sea water, and responsiveness to stimulation was tested by pinching the arm. Chemical stimuli, including dissolved amino acids, fish skin extract, conspecific skin extract, cephalopod ink, and sea water controls were perfused over the arm while nerve activity was recorded and arm movement was monitored by video. *D. pealeii* arms often failed to respond to pinch or chemical stimuli; when responses were detected, they were short lived, so insufficient data was collected to allow analysis. *O. bimaculoides* arms gave far more robust responses that were reliably evoked over a period of an hour or more. Arms consistently responded to fish skin extract, glycine, methionine, and conspecific skin extract with both nerve activity and reflexive motor actions. Notably, cephalopod ink was not detected by the arms. We conclude that chemosensory receptor cells on *O. bimaculoides* arms were able to detect environmentally relevant chemicals and even drive local motor responses. Although this preparation was not successful in *D. pealeii*, it worked well with *O. bimaculoides* and could be used to further explore chemosensory stimulus space and neural circuitry in octopus and, potentially, other cephalopods.

Disclosures: H.J. Rhodes: None. **K.E. Fouke:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.15/O41

Topic: F.01. Neuroethology

Support: Wellesley College Start-up Funds

Title: Dehydration state dependent alterations in humidity and visual perception across *drosophila* species

Authors: C. ZHU, I. D'ALESSANDRO, G. TURNER, *S. M. WASSERMAN;
Neurosci., Wellesley Col., Wellesley, MA

Abstract: Like humans, *Drosophila* must assign appropriate salience and subjective value to incoming sensory information in order to generate adaptive behavior. The salience and value assigned to a particular stimulus varies according to an animal's internal state, external state, and behavioral state. For example, the internal state dehydration, a constant threat to *Drosophila* due to their large surface area to volume ratio, must be accompanied by appropriate modifications to sensory perception that lead to the assignment of greater salience and positive value to water cues in order to generate water-seeking behavior. These responses may differ across species from varied habitats. *Drosophila mojavensis mojavensis* and *Drosophila mojavensis baja*, which inhabit water-scarce desert habitats, may exhibit different dehydration-state dependent responses to water cues compared to the generalist, *Drosophila melanogaster*. Using a virtual reality flight simulator that allows flies to rotate freely in response to controlled sensory stimuli, we show how dehydration differentially modifies humidity and visual perception in across *Drosophila* species. This work provides a foundation for determining how neural circuits in the brain can maintain enough flexibility to respond to a wide variety of contexts yet remain robust to reliably generate contextually appropriate behavior.

Disclosures: C. Zhu: None. **I. D'Alessandro:** None. **G. Turner:** None. **S.M. Wasserman:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.16/O42

Topic: F.01. Neuroethology

Title: Contributions of taurine and baclofen to subordinate female crayfish, procambarus clarkii

Authors: *C. M. MECCA, S. A. LISKOWICZ, R. F. WALDECK;
Biology/Neuroscience, Univ. of Scranton, Scranton, PA

Abstract: Taurine is a nonessential amino acid that is commonly consumed by millions in the form of energy drinks. Taurine is a known agonist for $GABA_A$ and $GABA_B$ receptors. Invertebrates, such as crayfish, are proposed to have similar GABAergic systems. Crayfish are excellent aggression models that will engage in agonistic battles to obtain resources and mates. The outcome of these conspecific fights determines the winner or loser status of the crayfish. By investigating the effects of taurine on crayfish aggression, we can gain insight on its role in subordinate behaviors such as the tail flip. Previous research that quantified the effects of taurine in losing, adult crayfish found that taurine submerged crayfish (25mg/L) showed a decrease in aggression accompanied by a significant increase of submissive behaviors such as tail flips ($p=0.02722$). This data suggests taurine may function similarly to GABA, thus producing an increase in tail flip frequency. To further investigate this hypothesis, extracellular electrophysiological recordings of nerve III (N3) on ganglion XII of the ventral nerve cord (VNC) were taken. The ganglion was administered either taurine (25mg/100mL), the GABA agonist Baclofen (50um/L), or vehicle in 0.5mL drip increments totaling 3mL. An increase in firing rate and a significant increase in number of peaks per stimulation ($p<0.0001$) was observed in both the taurine and Baclofen trials. These trends offer support to the increase in tail flip frequency observed in the behavioral trials and are critical in determining taurine's mechanism of action on the tail flip.

Disclosures: C.M. Mecca: None. **S.A. Liskowicz:** None. **R.F. Waldeck:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.17/O43

Topic: F.01. Neuroethology

Support: National Institute of Neurological Disorders and Stroke (R01-NS092716 B. Burrell)
NSF (DGE-1633213 B. Burrell)

Title: Repetitive nociceptive stimulation can produce habituating or sensitizing effects

Authors: *J. HOYNOSKI[1], B. D. BURRELL[2];
[1]Basic Biomed. Sci., [2]Basic Biomed Sci., Univ. of South Dakota, Vermillion, SD

Abstract: Nociceptors are sensory neurons that detect damaging or potentially damaging stimuli and directs that information to the central nervous system. Nociceptors can activate motor behaviors that lead to withdrawal or escape from painful stimuli, but they can also produce adaptations to painful stimuli by stimulating modulatory circuits. One type of adaptation is sensitization that protects the animal from additional damage and is partially mediated by nociceptive synapse potentiation. Another type of adaptation is habituation or a reduced behavioral response as a result of repeated stimulation. Habituation of defensive or evasive behavioral responses and their role in modulating responses to nociceptive stimuli are poorly understood. The mechanisms that mediate nociceptive habituation could be used to develop novel therapeutic approaches to treat chronic pain. Therefore, disruption of nociceptive habituation may contribute to chronic pain conditions suggesting that habituation may play a critical role in treating chronic pain. We address these questions using *Hirudo verbena* or the medicinal leech due to its well-described nervous system. In these studies, *Hirudo* is used to examine nociceptive habituation and the factors that modulate nociceptive habituation such as injury-induced sensitization and feeding states. Repetitive nociceptive stimulation produced two behavioral subpopulations, one that habituates to the nociceptive stimuli and the other that is dominated by efforts to evade the nociceptive stimuli via spontaneous withdrawals or locomotion. Current data suggests that injury-induced sensitization and feeding causes increased evasive response and decreased latency to habituation. Future experiments will further define the how endocannabinoids effect these behavioral outcomes.

Disclosures: **J. Hoynoski:** None. **B.D. Burrell:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.18/O44

Topic: F.01. Neuroethology

Title: Receptive field properties of visually sensitive interneurons coordinating light-guided behavior in the medicinal leech

Authors: T. K. H. GROVES, *J. A. JELLIES;
Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: Specialized visual systems are common across phyla, and although the laws of physics are universal, the structure of eyes in extant animals is highly diverse. Yet, basic components of visual systems (i.e. the neuronal circuits that analyze visual images) remain conserved—emphasizing the importance of understanding the systems responsible for driving light-guided behavior. In accordance with the central tenet of neuroethology, we seek to understand the strategies employed by neuronal circuitry underlying species-specific decision-making

processes. Light-guided behavior is driven by the capacity of visual systems to encode behaviorally relevant image features, such as spatial frequency and luminal and spectral contrast; however, many of the ways in which these features are encoded and decoded are not well understood. Here, we investigate these processes in the medicinal leech—a tractable annelid with simple eyes that can encode image features that drive light-guided behavior. One of these behaviors includes a mechanism for using color vision as a spatial cue, as leeches discriminate between green and UV light using dorsal/ventral spectral contrast to maintain body orientation.The leech detects light using and array of 10 cephalic eyecups and a distributed, segmentally iterated array of over 294 dermal sensilla positioned to survey a 3-D visual field. Our central hypothesis is that 2-D pixel arrays of nonimage-forming eyescan be used to extract low-resolution 3-D image features. We showed that adaptation and spectral response properties of the primary receptors in this distributed visual system are spatially mapped, and we are working to understand how this peripheral mapping cascades into the CNS to influence higher order executive functions. Preliminary work shows evidence of integrated responses in higher order interneurons (i.e. the S-cell). We extend this observation and hypothesize that interneurons involved in these light-guided behaviors are influenced by the contextual state of the animal as determined by visual inputs. To facilitate this aim we are identifying interneurons that receive visual input and evaluating how each responds to behaviorally relevant visual stimuli by using selective transections of afferent visual pathways. Synaptic interactions between sensory axons and even higher order command neurons may provide the substrates for synaptic computation for image feature extraction, and we also find that asymmetric responses in these interneurons reflect the asymmetric nature of some light-guided behaviors in the leech.

Disclosures: T.K.H. Groves: None. **J.A. Jellies:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.19/P1

Topic: F.01. Neuroethology

Support: NIDA - 1 P30 DA018310

Title: Identification of neurotransmitters that affect ciliolocomotor crawling in the sea slug Pleurobranchaea californica

Authors: *C. LEE[1], E. ROMANOVA[2], C. HUANG[3], J. V. SWEEDLER[4], R. GILLETTE[5]; [1]Neurosci. Program, Univ. of Illinois Urbana-Champaign, Urbana, IL; [2]Dept Chem., [3]Univ. of Illinois At Urbana Champaign, Urbana, IL; [4]Dept Chem., Univ. of Illinois at Chicago Dept. of Chem., Urbana, IL; [5]Dept Physiol., Univ. Illinois, Urbana, IL

Abstract: Gastropod mollusks, with simple nervous systems and large, identifiable neurons, have been used to gain critical insights into the neural regulation of behavioral hierarchies and decision making. An essential decision is that of whether or not to locomote, but the effect of other behaviors and internal state on locomotion remains relatively unstudied. Moreover, the neural bases of crawling in gastropods as a whole is not well understood compared to other behaviors (e.g. feeding, swimming, turning). Lastly, many gastropods, in particular nudipleuran sea slugs and pulmonates, crawl in large part via ciliary locomotion, where the myriad cilia on the foot paddle the animal through secreted mucus, a unique form of locomotion that adds further complexity to this behavior. To address this key gap in our understanding of the decision-making hierarchy in this taxon, we are studying the neural basis of crawling in the sea slug *Pleurobranchaea californica*. Here, we attempt to identify the neurotransmitters that control ciliary beating, to understand how crawling starts and stops. We combine untargeted mass spectrometry with systematic assays of small molecule neurotransmitters to search for possible neurotransmitters, a broad approach that should allow us to identify the various signaling molecules that affect the cilia, including previously uncharacterized ones. We find that the pedal nerves, which relay the signals for ciliary crawling, contain multiple putative neuropeptides, and that three of these molecules are released in response to nerve stimulation. Surprisingly, pedal peptide, which has been implicated in crawling in other species, was not among this group. Additionally, we confirm that application of serotonin causes a substantial increase in the rate of ciliary beating. Thus, there appear to be multiple neuromodulators that affect ciliary beating in *Pleurobranchaea*. This suggests that the decision to crawl in *Pleurobranchaea* is under complex neuronal regulation, and raises questions of how these different signaling molecules interact to control crawling.

Disclosures: C. Lee: None. **E. Romanova:** None. **C. Huang:** None. **J.V. Sweedler:** None. **R. Gillette:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.20/P2

Topic: F.01. Neuroethology

Support: NSF Grant 1818140

Title: Role of mechanoreceptors on magnetic field orientation by the nematode *C. elegans*

Authors: *C. BAINBRIDGE[1], T. OWOYEMI[1], K. OWOYEMI[1], B. PALUZZI[1], D. NISWONGER[1], Z. BENEFIELD[1], N. LEONARD[1], W. STEIN[1], D. HALL[2], A. VIDAL-GADEA[1];
[1]Illinois State Univ., Normal, IL; [2]Albert Einstein Col. of Med., Bronx, NY

Abstract: Many organisms, from bacteria to mammals, rely on magnetic fields to navigate their environment. While the list of magnetosensitive animals continually grows, the molecular mechanism underlying magnetic transduction remains unresolved. A favored model proposes that mechanoreceptors could accomplish this task by transducing the mechanical forces generated by associated magnetic particles as they are constantly pulled by the Earth's magnetic field.
C. elegans detects and orients to earth-strength magnetic fields. Using unbiased behavioral algorithms, we show that orientation to magnetic stimuli involves course corrections that align worms with the field vector, as well as additional reorientations that align the animal with its preferred migratory direction. At the cellular level, magnetic orientation requires the integrity of the AFD sensory neurons and their sensory endings.
After identifying magnetic particles adjacent to AFD's sensory endings, we turned to test the mechanoreceptor-magnetic particle model for magnetic transduction. We combined a Wormbot robot designed by the Kaeberlein lab with automatic behavioral tracking to conduct an RNA interference screen of all known mechanoreceptors and iron-handling genes in *C. elegans.* The cellular expression of genes required for magnetic orientation was determined by generating fluorescent reporter lines. The nematode *C. elegans* is uniquely positioned to contribute to the elucidation of the molecular basis of magnetic field transduction.

Disclosures: C. Bainbridge: None. **T. Owoyemi:** None. **K. Owoyemi:** None. **B. Paluzzi:** None. **D. Niswonger:** None. **Z. Benefield:** None. **N. Leonard:** None. **W. Stein:** None. **D. Hall:** None. **A. Vidal-Gadea:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.21/DP08/P3

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: F.01. Neuroethology

Support: NSF Grant 1704436

Title: Development of a robotic testing platform for neural control networks based on the common fruit fly (*Drosophila melanogaster*)

Authors: C. GOLDSMITH[1], ***N. S. SZCZECINSKI**[1], R. D. QUINN[2];
[2]Mechanical and Aerospace Engin., [1]Case Western Reserve Univ., Cleveland, OH

Abstract: Potential for a high degree of mobility has made legged robots the continued focus of robotic design for a variety of exploratory and autonomous applications; however, the

complexity involved in creating a robust and adaptable network to control locomotion stands as the greatest hurdle in implementing legs for more complex terrains. Biologically inspired robotics shows promise using naturally existing physiology and neurobiology to solve this problem, with animals such as insects providing excellent physical models for legged locomotion over difficult terrains. Fruit flies in particular prove interesting for biologically inspired robotics due to the abundance of genetic tools available to manipulate their nervous systems. Experiments utilizing these tools have huge potential to thoroughly explain the neural control of walking in insects, so to more directly apply findings regarding fruit fly locomotion to control of walking machines, we have developed Drosophibot, a hexapod robot with several key fruit fly characteristics such as a insect-like dynamic scaling, compliant tarsus-like feet, and a retractable abdominal segment for more animal-like weight distribution. The robot additionally includes strain gauges along the femur and tibia to provide sensory feedback similar to sensory organs such as the campaniform sensilla in the insect, providing a necessary analog for implementation of such feedback on the platform.
Our goal is to use data from neuroscience to construct a bottom-up, dynamical model of the sensorimotor pathways that contribute to insects' posture and locomotion. We have begun developing such "synthetic nervous system" models for position and velocity sensory feedback of *Drosophila* in the femur-tibia (FTi) joint for use on Drosophibot, to both implement low-level control mechanisms for locomotion on the robot and to observe the closed-loop behavior of the joint where previously only open-loop has been recorded (Hellekes et al., 2011). We have found it straightforward to implement identified reflex pathways (Sauer et al., 1996) using our modeling approach, and this work shows promise for controlling the desired behaviors on Drosophibot. The development of this network will aid in our eventual goal of modeling *Drosophila's* nervous system in its entirety on Drosophibot, to yield biologically accurate locomotion.

Disclosures: C. Goldsmith: None. **N.S. Szczecinski:** None. **R.D. Quinn:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.22/P4

Topic: F.01. Neuroethology

Support: NSF: IOS-1557913
Esther A & Joseph Klingenstein Fund, Inc.
Richard and Susan Smith Family Foundation

Title: The antennal lobe is a *drosophila melanogaster* locus of behavioral individuality

Authors: *M. A. CHURGIN, B. L. DE BIVORT;
Harvard Univ., Boston, MA

Abstract: Behavioral variation persists even in isogenic animals reared in identical environments. This is especially surprising in animals with nominally stereotyped nervous systems. Importantly, neurodevelopmental disorders such as autism result from pathological idiosyncrasy in neuronal wiring and function. Therefore, it is crucial to gain a fuller view of how nervous systems vary and how this variation affects behavior. Fruit fly olfaction is a powerful model for investigating the neural bases of individuality owing to its well-studied and highly stereotyped nature. Olfactory receptor neurons synapse on projection neurons (PNs) in ~50 glomeruli in the antenna lobe (AL). Recent work has uncovered subtle idiosyncrasies in AL wiring and activation, suggesting this circuit does indeed vary between individuals. Furthermore, individual flies display persistent individual odor preferences (day 1 to day 2, r=0.37, n=50 flies). Which elements of the olfactory circuit explain these varying behavioral preferences in such a highly stereotyped system?
To answer this question, we performed paired behavior and optical physiology experiments in individual flies expressing a calcium reporter only in PNs (GH146>GCaMP6m) or ORNs (Orco>GCamp6m). We measured odor preference by tracking flies in individual tunnels and filling each half of the tunnel with one of two odors (MCH or OCT). Individual preference was determined as the proportion of time spent in each half of the tunnel. We then performed volumetric two-photon imaging in each individual to record PN or ORN responses to an odor panel. We performed principal component analysis on glomerular activations to compare flies in coding space and found that distances are lower within-flies (trial to trial) than across-flies, a sign of individuality in odor coding. We then generated a linear classifier from individual principal component scores to predict individual preference and found that PN, but not ORN, dynamics could explain behavioral variation (n=47 flies, R^2=0.21). We confirmed that this PN model could predict behavior on held-out test data (n=22 flies, R^2=0.3). Finally, we conducted a power analysis which takes into consideration behavioral variation, and we conclude that the majority of behavioral variation can be explained by idiosyncrasies in PN dynamics.
Identifying the sources of neural variability is critical to increasing our understanding of both normal and pathological neurological function. The results described here may shed light on how behavioral individuality can emerge from a nominally stereotyped network through idiosyncrasies in neural coding.

Disclosures: M.A. Churgin: None. **B.L. de Bivort:** None.

Poster

067. Invertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 067.23/P5

Topic: F.01. Neuroethology

Support: NSF CAREER

Title: Regulation of *Drosophila* courtship behavior and neuronal development by the dissatisfaction nuclear receptor

Authors: *J. DUCKHORN[1], J. CANDE[3], D. STERN[3], T. SHIRANGI[2];
[1]Biol., [2]Villanova Univ., Villanova, PA; [3]Janelia Res. Campus, Ashburn, VA

Abstract: How genes build the neural circuits that underlie innate animal behaviors is poorly understood. During courtship, *Drosophila* males court females with a series of elaborate innate behaviors, whereas females decide whether or not to mate. Mutations in the *Dissatisfaction* gene, which encodes a developmental nuclear receptor, cause sex-specific courtship abnormalities in both sexes. To understand how *dsf* influences the neural circuits that underlie courtship, we sought to identify the neurons that express *dsf* in the fly brain that contribute to courtship. Here, we find that *dsf* is expressed in several subsets of neurons in the fly nervous system of both sexes. We identify a small subset (i.e., 3+) of *dsf*-expressing neurons in the fly's abdominal nervous system that contribute to most behaviors that are altered in *dsf* mutant males and females. Loss of *dsf* function causes sex-specific anatomical phenotypes in these neurons, suggesting that *dsf* regulates neuronal development and courtship behavior by functioning with the sex determination pathway in flies. This work provides insights into how a developmental gene patterns the circuits for an innate animal behavior.

Disclosures: J. Duckhorn: None. **J. Cande:** None. **D. Stern:** None. **T. Shirangi:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.01/P6

Topic: F.01. Neuroethology

Support: EPSRC Grant EP/P030203/1

Title: Describing whisker shape and mechanics in order to better understand tactile sensory signals across different individuals and species

Authors: *R. A. GRANT[1], G. DOUGILL[1], E. L. STAROSTIN[2], G. H. M. VAN DER HEIJDEN[3], V. G. A. GOSS[2];
[1]Manchester Metropolitan Univ., Manchester, United Kingdom; [2]London South Bank Univ., London, United Kingdom; [3]UCL, London, United Kingdom

Abstract: One of the most striking features on the faces of many mammals are the presence of their long whiskers, or vibrissae. Primarily, these are used for touch sensing, and can be employed to guide behaviours such as foraging, navigation, and social interactions. Whiskers are thick, tapered hairs that are enclosed by an innervated follicle. The size and shape of each whisker will influence the tactile signals that reach the follicle and are transmitted to the brain. Whiskers are a model of sensory processing; however, our understanding of this system relies on the accuracy by which mathematical models capture the forces of the whisker, which also includes approximating their shape. Typically, whiskers have been modelled as a parabola based on Cartesian coordinates of the whisker centreline. However, different parabolas have been suggested for different species, making comparisons challenging. We suggest that a model based on whisker length, independent of its position and orientation, is more appropriate, especially for comparative studies. Indeed, in our study, we show that Euler spirals provide convenient mathematical models for analytical studies of the mechanics of intrinsically curved rods, as opposed to expressing that shape in terms of Cartesian coordinates. We will present data from 2-dimensional whisker scans and 3-dimensional Micro CT scans of mystacial pads, from a variety of individuals in rats, and from over twenty different mammalian species. We will demonstrate that the mathematically simple model of linear curvature (the Euler spiral) is sufficient to fit our measured data and can account for a wide range of vibrissae morphologies including increasing, decreasing and inflection curvatures. We show that whisker shape is fairly consistent across species, but that whiskers are especially long in nocturnal, arboreal and aquatic mammals that also tend to move their whiskers. Understanding more about differences in whisker shape and mechanics will provide important insights in to mammalian sensory biology and sensory processing.

Disclosures: R.A. Grant: None. **G. Dougill:** None. **E.L. Starostin:** None. **G.H.M. van der Heijden:** None. **V.G.A. Goss:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.02/P7

Topic: F.01. Neuroethology

Support: James S. McDonnell Foundation 22002078
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South African National Research Foundation

Title: Comparative neocortical neuromorphology in felids: African lion (pantera leo), African leopard (panthera pardus pardus), and cheetah (acinonyx jubatus)

Authors: *B. G. JACOBS[1], **V. NGUYEN**[1], R. UCHIDA[1], A. WARLING[1], L. SLOAN[1], C. DODELSON[1], R. SHIN[1], B. WICINSKI[2], M. BERTELSEN[3], C. D. STIMPSON[4], M. A. SPOCTER[5], M. SCHALL[1], P. HOF[2], C. SHERWOOD[4], P. MANGER[6];
[1]Colorado Col., Colorado Springs, CO; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Copenhagen Zoo, Fredericksberg, Denmark; [4]George Washington Univ., Washington, DC; [5]Dept. of Anat., Des Moines Univ., Des Moines, IA; [6]Univ. of the Witwatersrand, Johannesburg, South Africa

Abstract: The present quantitative study extends recent investigations in non-domestic felids by examining neuronal morphology in the neocortices of the African lion (*Panthera leo*), African leopard (*Panthera pardus pardus*), and cheetah (*Acinonyx jubatus*). Tissue samples were removed from prefrontal, motor, and visual cortices, and stained with a modified rapid Golgi technique. A total of 652 neurons were quantified using computer-assisted morphometry. Neurons in all species except the lion were sufficiently impregnated for accurate quantitative dendritic measurements. Despite poor stain quality in the lion tissue, descriptions of neuronal morphology were still possible. Qualitatively, the range of spiny and aspiny neurons across the three species was morphologically consistent with that observed in other felids (e.g., Siberian tiger, clouded leopard; Johnson et al., 2016), with typical pyramidal neurons being the most prominent neuronal type. Quantitatively, somatodendritic measures of typical pyramidal neurons in the cheetah were generally larger and more complex than those in the African leopard despite similar brain sizes. A MARsplines analysis of dendritic measures correctly differentiated 87.4% of complete typical pyramidal neurons between the African leopard and cheetah. Consistent with Jacobs et al. (2018), motor gigantopyramidal neurons were disproportionately large across all three felids, possibly due to specializations in their musculoskeletal systems and hunting behavior. The large size of these neurons in the cheetah, which unlike lions and leopards, does not belong to the *Panthera* genus, suggests that motor gigantopyramidal neurons evolved independently in these divergent felid species.

Disclosures: B.G. Jacobs: None. **V. Nguyen:** None. **R. Uchida:** None. **A. Warling:** None. **L. Sloan:** None. **C. Dodelson:** None. **R. Shin:** None. **B. Wicinski:** None. **M. Bertelsen:** None. **C.D. Stimpson:** None. **M.A. Spocter:** None. **M. Schall:** None. **P. Hof:** None. **C. Sherwood:** None. **P. Manger:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

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Topic: F.01. Neuroethology

Support: JP25116526

JP15H01297
JP16H06462
Astellas Pharma Inc.

Title: Population coding of multiple types of information in dentate gyrus

Authors: *T. MURANO[1], R. NAKAJIMA[1], A. NAKAO[2], S. AMEMORI[3], N. HIRATA[4], A. MURAKAMI[2], Y. KAMITANI[5], J. YAMAMOTO[6], T. MIYAKAWA[1];
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Abstract: The hippocampal dentate gyrus (DG), in which neurons have highly sparse and diverse response properties, is thought to contribute to the multiple functions, such as place recognition, exploration, and pattern separation. However, it remains mostly unknown how DG neurons are involved in these functions. In this study, by using Ca2+ imaging in freely exploring mice and machine learning methods, we investigated what types of information are represented in the population activity of dorsal DG and how each DG neuron contributes to the population coding.
Our results demonstrate that multiple types of information could be decoded accurately from the population activity of DG neurons, position/velocity in open field test, and current/future location in the T-maze test. In the open field test, there were four groups of DG neurons that carry information about position, velocity, both position and velocity, and neither of them. In the T-maze test, there were also four groups of DG neurons with information about current location, future location, both current and future locations, and neither of them. These results indicate that the population activity of DG neurons encodes multiple types of information and each DG neuron contributes to a different extent in information coding.
We performed the same analysis on the alpha-isoform of CaMKII heterozygous knockout (αCaMKII+/-) mice, which shows psychosis-like behavior. In the open field test, decoding accuracy of the position, not velocity, was significantly lower than that of wild-type mice. In the T-maze test, decoding accuracy of the future location, not the current location, was also significantly lower than that of wild-type mice. These results showed that a specific type of information could be selectively impaired in the population activity of DG neurons in αCaMKII+/- mice. These results also suggest that different types of information could be distributed in different ensembles of DG neurons.
In conclusion, we revealed that multiple types of information, position/velocity in open field test and current/future location in the T-maze test, are represented in the population activity of DG neurons. Also, we found that these types of information are encoded by different populations of DG neurons with an overlap.

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Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.04/P9

Topic: F.01. Neuroethology

Support: CIHR (MOP-102662)
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FRQS

Title: Resynthesizing the functional organization of the primate brain according to its evolutionary history

Authors: *P. CISEK;
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Abstract: The functional organization of the primate brain is often described according to information processing functions, including perceptual processes such as object recognition, cognitive processes such as decision-making, and motor processes such as trajectory execution. While these are useful for defining empirical questions that theories seek to address, the resulting explanations often do not naturally map to circuits in the brain. An alternative approach to subdividing the functional organization of the brain is to follow its evolutionary history, constrained by comparative and developmental data on how specific circuits and functions gradually differentiated and specialized over evolutionary time. Here, I will present a hypothetical sequence of functional innovations along the lineage that leads from early multicellular animals to primates, emphasizing the gradual extension of feedback control circuits to more effectively interact with the external world. Key steps include the elaboration of simple chemotaxis to hypothalamic foraging strategies governed by tonic dopamine; the emergence of oriented escape and approach behaviors in the tectum / colliculus; the expansion of the alar hypothalamus into the telencephalic pallium and subpallium (basal ganglia) capable of reinforcement learning; the specialization of systems for local exploitation (ventrolateral pallium) versus long-range exploration (medial pallium / hippocampus); the expansion of the dorsal pallium into the mammalian neocortex, including dorsomedial streams for specifying species-typical actions (fronto-parietal cortex) and ventrolateral streams for key stimulus detection and valuation (temporal and orbitofrontal cortex); and the primate-specific expansions of the prefrontal, parietal, and temporal neocortex that support arboreal foraging and more sophisticated social interactions. The resulting functional architecture captures many well-known features of the primate brain (e.g. crossed and uncrossed pathways, corticostriatal loops, dorsal vs. ventral visual streams, etc.) as well as some neurophysiological results that are difficult to interpret in terms of classical information processing functions (e.g. the distribution and mixtures

of sensory, motor, and cognitive variables across cortical and subcortical circuits, the time-course of activation across different regions, etc.). It is suggested that an evolutionary approach such as this can yield a more natural framework for interpreting neural activity and building computational models of behavior.

Disclosures: P. Cisek: None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.05/P10

Topic: F.01. Neuroethology

Support: Nc3Rs David Sainsbury Fellowship

Title: Robust reconstruction of mouse poses

Authors: *S. STORCHI;
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Abstract: Quantifying changes in mouse postures as a function of changes in the visual scene is a critical step for understanding visual processing and learning. However a robust method to detect postures from specified body landmarks in freely moving animals is currently missing. Here we propose a solution to this problem by combining convolutional neural networks, 3D reconstruction from multiple camera views and active shape models. First we use convolutional networks to estimate body landmarks from individual cameras. From these landmarks we then generate a set of candidate 3D reconstructions of the mouse postures. Finally these postures are scored and regularised by using active shape models to generate a single robust estimate of the mouse posture. We apply this algorithm to a dataset of innate and conditioned behavioural responses to a variety of behaviourally salient stimuli. Our results show that the proposed algorithm, combined with time series segmentation and classification allows for extraction of a remarkable diversity of visually evoked behaviours.

Disclosures: S. Storchi: None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.06/P11

Topic: F.01. Neuroethology

Support: MN Futures

Title: A novel method for camera-based pose tracking of rhesus macaques in an open enclosure

Authors: *B. Y. HAYDEN[1], B. R. EISENREICH[2], P. BALA[1], Y. JAFARIAN[1], H. PARK[1], J. ZIMMERMANN[1];
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Abstract: The neuroscience of behavioral decision-making in rhesus macaques is generally studied using laboratory contexts in which motion is limited. An alternative approach is to allow for unrestrained movement, monitor body position, and infer decisions from pose information. We developed a novel markerless pose estimation system that we call Skeletor. Our system makes use of 62 machine vision cameras organized in two rows that surround a 9'x9'x8' cage that affords free movement. The cameras are mounted on an exoskeleton frame mounted around the perimeter of the cage. Cameras are synchronized via a digital pulse at 30 Hz and transmit their datastream to six custom built central processor systems. We leverage the synchronized multiview image streams to reconstruct body landmarks on macaque subjects in 3D. For each image, a convolutional neural network (CNN) is used to localize 13 body landmarks based on visual appearance. A main challenge of training such CNN lies in obtaining a large training set. Unlike existing large human datasets that have been annotated by crowd workers, collecting a comparable dataset for macaques is infeasible due to the requirement of expert knowledge and large intra-class variation. We therefore instead use a semi-supervised learning approach that enables us to explore the unlabeled data using multiview geometry in conjunction with a small set of professionally annotated data. The key insight is that it is possible to geometrically transfer the annotations in one image to the other view images without additional manual efforts (cross-view self-supervision). This allows us to propagate visual information across views and time. We demonstrate a strong recognition performance of the CNN trained by less than 4% of annotated data. Given the landmark recognition on multiview images, we triangulate them to produce 3D landmarks given estimated camera parameters (orientation and translation). In addition to 3D landmark localization, we build a volumetric representation of individual subjects to estimate detailed pose using a 3D visual hull algorithm based on semantic segmentation. Together this approach allows us to reliably reconstruct macaques poses in our freely behaving environment.

Disclosures: B.Y. Hayden: None. **B.R. Eisenreich:** None. **P. Bala:** None. **Y. Jafarian:** None. **H. Park:** None. **J. Zimmermann:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.07/P12

Topic: F.01. Neuroethology

Title: A hypothetical model of sensorimotor adaptation in larval zebrafish

Authors: *K. J. HERRERA, F. ENGERT;
Harvard Univ., Cambridge, MA

Abstract: In the present study, we use the larval zebrafish as a model to examine the capabilities of fish to sense and encode the ionic content of their environment. As a freshwater fish, zebrafish cannot tolerate high salt environments. Therefore, we predicted that zebrafish possess neural mechanisms that enable the avoidance and navigation of salt gradient. We find determine that zebrafish can avoid and thus detect salt. In particular, they respond to temporal increases in salt by increasing their reorientation probability. We then use calcium imaging techniques to describe the systems responsible for absolute and relative salt detection. We finding that the lateral line and olfactory systems largely encode absolute salinity concentrations by detecting small cations. Meanwhile, changes in salinity are represented in the hindbrain populations of neurons with different dynamics. Most notably, inhibitory neurons with time constants up to a minute long allow the fish to dynamically adjust their estimate of baseline salinity.

Disclosures: K.J. Herrera: None. **F. Engert:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.08/P13

Topic: F.01. Neuroethology

Support: Conacyt-415154

Title: Litter size influence impulse propagation and myelination of axons in cutaneous nerves of the rat

Authors: *V. MARTINEZ-ALVAREZ[1], B. SEGURA-ALEGRIA[2], E. E. RODRIGUEZ-TORRES[3], M. PORRAS[4], E. AGUIRRE-BENITEZ[4], V. RAMIREZ-ROSAS[1], I. JIMÉNEZ-ESTRADA[1];
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Abstract: In a previous study, we have shown a significant decrement in the amplitude of the compound action potential (CAP) evoked in sural nerves of artificially reared rats (AR) as compared with that of nerves from mother reared rats. Additionally, AR nerves shown a decrement in myelin sheath thickness. However, those effects were prevented by the presence of littermates during AR (Segura *et al*., Dev. Neurobiol. 74(12): 1184, 2014). The latter is indicative of the importance of littermates on the development of cutaneous nerves. In this study, we analyze the effects evoked by the interaction of siblings reared in litters of different size on the histological and electrophysiological characteristics of the sural nerve in the male rat. We use litters culled with one puppy rat from the 5th postnatal day (sibling deprivation) and litters culled with 3, 6, 9 y 12 pups from birth to the experimental day (PN60). Morphometric parameters were determined during pre- and post-weaning periods and milk consumption was calculated at PN10. At PN60, the left sural nerve was exposed and prepared for recording of CAP and their threshold (T), peak amplitude (pA), area (A) and conduction velocity (CV) were determined. Semi-thin slices (0.5µm) from the contralateral sural nerves were obtained and stained with standard histological techniques (Toludine blue stain), photographed and the transversal diameter and area the myelin sheet of axons (n=250) were randomly measured. So far, our results indicate that puppies from 3, 6 and 9 litters showed significant higher weight increments and milk consumption than those from of 1 and 12 litters puppies during the pre-weaning period. Histological analysis reveals that the thickness of the myelin sheath follows the next sequence: (6 sibs,3 sibs,9 sibs, sibling deprivation =12 sibs). However, the transversal area and axon diameter did not present differences. On the other hand, electrophysiological records reveal that the amplitude and area of CAP show a similar sequence observed in the thickness of the myelin sheath (6 sibs,3 sibs,9 sibs, sibling deprivation =12 sibs). In conclusion, our data support the idea that sibling interaction during the pre-weaning period influences the myelination and electrophysiological proprieties in adult sural nerve.

Disclosures: V. Martinez-Alvarez: None. **B. Segura-Alegria:** None. **E.E. Rodriguez-Torres:** None. **M. Porras:** None. **E. Aguirre-Benitez:** None. **V. Ramirez-Rosas:** None. **I. Jiménez-Estrada:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.09/P14

Topic: F.01. Neuroethology

Support: NIH Grant DC012813

Title: Magnetosensory neurons encode direction in gravity centered coordinate system

Authors: *J. D. DICKMAN[1], N. A. LEFELDT[1], E. BERTRAM[3], H. ADAMS[3], D. MCDONALD[3], L.-Q. WU[2];
[1]Neurosci., [2]Baylor Col. of Med., Houston, TX; [3]Rice Univ., Houston, TX

Abstract: Many animals rely on the Earth's geomagnetic field for spatial orientation and navigation. Our previous work identified neurons in the pigeon brainstem that encode direction and magnitude of generated magnetic fields that are comparable to the omnipresent geomagnetic field. Here, we report that magnetic response (MR cells) neurons have individual preferred directions that are each referenced to the allocentric constant, gravity. Pigeons were implemented with plastic head mounts, recording grids, and body holders within a DC field coil system (Merritt). The field coil system could generate magnetic fields in any spatial direction at intensities ranging between 0.0 - 2 Gauss. The animal was placed in a plastic body manipulator within the coil system that could vary head fixed/body position in 3D independently inside the magnetic field. The entire system was mounted on a hexapod hydraulic motion platform in an electromagnetic shielded room. Neural recordings were performed with high impedance, non-magnetic, tungsten electrodes and standard neurophysiological technique. Eight four neurons from 12 pigeons were responsive to an applied magnetic field (Merritt coil) while over 400 other neurons in the same regional area of the brainstem were not. All 84 cells were additionally responsive to linear acceleration, but not to rotational acceleration. Thirty one of the 84 MR cells were also tested with different head positions relative to a gravity fixed magnetic coil system. All were found to have preferred response directions (PD) that were consistent relative to gravity, independent of head orientation in pitch and/or roll position. In contrast, as a control, head position was varied in the azimuth (constant relative to gravity) and all 31 PDs shifted by an equal amount to the new yaw head direction. In addition, 3D directional sensitivity thresholds were calculated (signal detection theory) for the PDs and found to vary between 1.5 - 7.7 degrees in magnetic space for 29/31 MR cells, with an additional 2 MR cells exhibiting thresholds of 15.2 and 34.7 degrees, respectively. Our findings demonstrate that the neural substrate for avian magnetoreception operates in a world-fixed reference frame with sufficient directional sensitivity for geomagnetic navigation.

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Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.10/P15

Topic: F.01. Neuroethology

Title: The evolution of esthetics in goal-directed foraging

Authors: *E. D. GRIBKOVA, R. GILLETTE;
Neurosci. Program, Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: The esthetic sense, at the core of creative endeavor and appreciation in science and art, arises from affective valuations based on positive or negative feelings. Our evidence from a simple invertebrate predator indicates a likely origin of the esthetic sense in the neuronal circuitry of decisions underlying foraging. Behavioral choice in foraging is governed by reward learning and motivation, which interact to assign subjective value to sensory stimuli in the environment. These qualities characterize opportunistic, foraging generalists that hunt in unpredictable environments, and they are the foundation of esthetics. The esthetic sense in animals allows them to make value judgements based on learned and established preferences. While these preferences are quite simple in foraging generalists like the sea-slug *Pleurobranchaea californica*, they can be traced to increasingly complex creative syntheses in niche modification and elaborate nest-building in vertebrates and arthropods, in particular.
Using the agent-based foraging simulator, ASIMOV, we explore emergence and evolution of the esthetic sense in a simple foraging generalist, through the addition of three capacities:
1) Enhanced exteroceptors and attentional mechanisms.
2) Physical handling of objects in the environment.
3) Spatial and episodic memory.
Olfaction is used for both odorant discrimination and spatial navigation. To map spatial odor and temporal representations, we devised an “associations matrix” to memorize and replay sequences. It uses reinforcement learning and hippocampus-like sequence learning to map and establish relations. The associations matrix is much simpler than most models of hippocampal function that use realistic spiking conductance-based neuron models. However, it accomplishes the same basic computational tasks.
The enhanced abilities of the forager are shown in use of its esthetic sense to map its territory, physically alter its environment to construct a home burrow, and navigate its environment to selectively forage and return home. In an analog of the Morris water maze, the forager is placed in an aversive environment with two or more odor source landmarks. The landmarks are used to

efficiently learn to locate a “hidden” odorless target for escape. Addition to ASIMOV’s forager of mechanisms for focused attention, object handling, and spatial and episodic memory shows how the neuronal circuitry of foraging decision can serve as the framework for elaborating the esthetic sense in evolution.

Disclosures: E.D. Gribkova: None. **R. Gillette:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

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Youth Innovation Promotion Association CAS 2017413(P.W.)

Title: Neural circuits of distinct defensive-arousal states evoked by visual threat stimuli

Authors: *Z. REN, N. LIU, K. HUANG, Y. TIAN, Q. YANG, J. ZHANG, X. RONG, Y. TIAN, F. JU, P. WEI, L. WANG;
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Abstract: There are many kinds of threat-induced states, such as orienting and freezing. Both of them share an immobility and are therefore difficult to tell apart. Here, using different types of visual stimuli, we observed the two defensive states and investigated the underlying neural basis. Using multidisciplinary methods, we found that neurons in superficial and intermediate layers of superior colliculus (SC) have distinct molecular identities, divergent final targets through lateral posterior nucleus (LP) and different functional circuits. One pathway from SC to LP processes the orienting behavior and mediates transient elevation of arousal state. While the other corresponds to the freezing behavior and relates to a violent and long lasting increase of arousal. These authors contributed equally: Z.R., N.L., K.H.

Disclosures: **Z. Ren:** None. **N. Liu:** None. **K. Huang:** None. **Y. Tian:** None. **Q. Yang:** None. **J. Zhang:** None. **X. Rong:** None. **Y. Tian:** None. **F. Ju:** None. **P. Wei:** None. **L. Wang:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Wellcome Trust 200501/Z/16/Z
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DFG SFB 1233
Robust Vision 276693517

Title: Quantifying the predictability of rat behavior

Authors: *E. MENICHINI[1], T. MUZZU[1], K. JAIN[2], J. H. MACKE[4], G. BERMAN[3], A. B. SALEEM[1];
[1]Exptl. Psychology, Univ. Col. London, London, United Kingdom; [2]Physics, [3]Biol., Emory Univ., Atlanta, GA; [4]Electrical and Computer Engin., Tech. Univ. of Munich, Munich, Germany

Abstract: When freely interacting with the environment, animals display richly-structured behavioral sequences with complex temporal dynamics. These sequences are often repeated in a stereotyped manner and can be classified into distinct behavioral motifs. Thus, if these motifs can be combined into longer time scale dynamical structures, we should be able to measure signatures of predictability that persist long into the future. These time scales have been hypothesized to have a hierarchical structure that links postural movements to the emergence of complex behavioral patterns. In this study, we quantified the behavioral dynamics of freely-moving rats, assessing how the predictability and hierarchy of their behaviors may be linked and shaped by context: familiarity or light-dark conditions.
We recorded videos of four rats as they explored an initially-novel environment (~1 m^2), enriched with objects and food, across multiple three hour sessions. The first session was in the dark for half of the animals, and we alternated between light and dark conditions across sessions. We identified specific features of the animal's body (e.g. snout, ears, tail) offline using DeepLabCut (Mathis et al., 2018), a deep-learning based tracking tool, which robustly tracked the animal features in both light conditions. In order to quantify the behavioral repertoire of the animals, we first mapped the high-dimensional postural data onto a two-dimensional space using MotionMapper (Berman et al., 2014). We then segmented the resulting behavioral density map to identify behavioral states and quantify their predictability under different contexts.

The animals actively explored the environment for 15-30% of the time during individual sessions. Across sessions, exploration time decreased as the environment became more familiar. In the first session, the animals in the dark ran at higher speed compared to animals in the light. Animals showed stereotyped behaviors for 50-60% of the time within individual sessions, and we identified multiple stereotyped behavioral states. Consistent with previous observations in *Drosophila* (Berman et al., 2016), we find that behavior is organised hierarchically and that transitions between states are predictable at long time scales, pointing to predictability as a potential organizing framework to understand behavior across animals, contexts and species.

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Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.13/P18

Topic: F.01. Neuroethology

Title: Newts can learn but are not humdrum

Authors: A. RETAILLEAU[1], ***T. BORAUD**[2];
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Abstract: Decision-making is the process of identifying and choosing alternatives based on the values, preferences and beliefs of the decision-maker. It implies cortex, thalamus and basal ganglia (BG) but the respective role of each structure is still debated. Study of decision-making process in mammals is hampered by the over representation of the cortex. Urodeles (group of amphibians) offer a remarkable opportunity to address this question. The organization of the BG in these vertebrates is similar to mammals but their cortex lacks the capability to perform selection. Thus, urodeles are a unique model to study the functional role of BG in decision-making process irrespective of the role of the cortex. We used an aquatic T-Maze where Spanish ribbed newt (*pleurodele Waltl*) had to discriminate between two cues in order to get a reward (shade). Our results showed that newts learn to associate a cue to the reward and optimize their choices in order to get it. However, our results highlight that these vertebrates are not able to automatize their choice. When the cues are switched, the animals are able to learn quickly the new rule without showing any habitual behavior related to the older rule. This result suggests that BG are necessary for response learning but not sufficient for the formation of habits. This study provides the first glimpse of decision-making process in a cortexless specie. More investigations will be needed to decipher the selective role of the subpallium (striatum) in the decision-making process.

Disclosures: **T. Boraud:** None. **A. Retailleau:** None.

Poster

068. Vertebrate Sensory-Motor Integration

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Support: BBRF NARSAD
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Title: Cell type specific membrane potential changes in dorsolateral striatum accompanying sensorimotor learning

Authors: *T. SIPPY[1], C. N. CHAIMOWITZ[2], S. CROCHET[3], C. C. PETERSEN[4];
[1]Psychiatry and Neurosci., [2]New York Univ., New York, NY; [3]EPFL, Lausanne, Switzerland;
[4]École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: The dorsal striatum integrates sensorimotor and motivational signals playing a key role in reward-based learning of goal-directed behavior. Although it is widely thought that cortico-striatal plasticity is important for linking sensory inputs to goal directed actions, *in vivo* evidence to support this is scant. In addition, the cell-type-specific mechanisms underlying reinforcement learning remain unknown. Using whole cell recordings in awake behaving mice, we investigated changes in membrane potential dynamics (V_m) of striatal neurons as mice learned to lick a reward spout in response to whisker deflection. Task learning was accompanied by an increased early sensory-evoked excitation specifically in dopamine-receptor type 1-expressing direct pathway striatonigral neurons (dSPNs), but not in dopamine-receptor type 2-expressing indirect pathway striatopallidal neurons (iSPNs). At later post-stimulus times, both striatonigral and striatopallidal neurons were more depolarized in Expert compared to Naïve mice. In contrast, tonically active, putative cholinergic, striatal neurons (TANs) acquired a hyperpolarizing response with learning, driving a pause in firing. Our membrane potential data reveal cell-type-specific changes in striatal function accompanying sensorimotor learning, consistent with the hypothesis that dopamine signals potentiate reward-predicting sensory input onto striatonigral neurons, thus causally contributing to task learning.

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Poster

068. Vertebrate Sensory-Motor Integration

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.15/P20

Topic: F.01. Neuroethology

Support: Simons Foundation Award 543015SPI

Title: Mice exhibit one-shot learning when navigating a maze

Authors: *M. H. ROSENBERG[1], T. ZHANG[1], P. PERONA[2], M. MEISTER[3];
[1]Div. of Biol. and Biol. Engin., [2]Div. of Engin. and Applied Sci. & Tianqiao and Chrissy Chen Inst. for Neurosci., [3]Div. of Biol. and Biol. Engineering&Tianqiao and Chrissy Chen Inst. for Neurosci., Caltech, Pasadena, CA

Abstract: *Background*: How do animals learn from only a handful of experiences? Despite advances in machine learning and systems neuroscience, existing theories remain unable to explain how biological organisms learn from just a few relevant experiences. Efforts to better characterize 'rapid learning' have been hampered both by the absence of a known synaptic learning rule and by technical difficulties in quickly training model organisms to perform even simple experimental tasks. Much research in learning and decision making involves rodents that are trained laboriously on seemingly trivial tasks. Even a simple binary choice task can require thousands of trials of training and often results in only mediocre performance. Clearly, learning and decision-making in the real world must happen faster and more efficiently. *Approach*: To observe learning and decision-making under more natural conditions we allowed mice to explore a complex maze in search of a water reward. This classic behavioral tool was complemented with a computer vision system, automated analysis of animal trajectories, and computational modeling of behavior. *Results*: We find that mice acquire in under 8 hours sophisticated maze navigation behaviors, requiring the maintenance of at least 6 bits of task information complexity. In particular, some mice can learn and remember a complex trajectory (6 correct turns) after a single encounter with the water reward. By analyzing their behavior before and after that formative experience we aim to evaluate different theories of learning. Some evidence speaks against the conventional models of reinforcement learning. *Conclusion*: In an ethologically inspired behavioral paradigm mice display remarkable abilities at one-shot learning of complex decision strategies.

Disclosures: **M.H. Rosenberg:** None. **T. Zhang:** None. **P. Perona:** None. **M. Meister:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.16/P21

Topic: F.01. Neuroethology

Title: Homeostatic plasticity mechanisms in nature: Increased excitability of motor neurons after months of inactivity in a hibernating frog

Authors: *J. M. SANTIN;
Univ. of North Carolina At Greensboro, Greensboro, NC

Abstract: Neural systems must keep their output in a set range to generate stable behaviors. Compensatory mechanisms that oppose disturbances in activity- broadly viewed as "homeostatic"- are a common feature that neural systems use to achieve this end. Most studies to date have used brute-force experimental approaches (injury, drugs, and genetics) to push neurons outside their normal activity ranges to test if homeostatic mechanisms can correct their function. Instead, I take the opposite approach and ask if a nervous system that experiences highly variable activity levels caused by challenges in the natural environment uses homeostatic mechanisms to control its output. American bullfrogs are interesting in that they do not breathe air for months at a time when hibernating under water, yet surprisingly, motor networks that produce breathing work as usual immediately after months of silence (Santin & Hartzler, 2016, *J. Exp. Biol.*). This suggested to me that homeostatic changes may compensate for inactivity and regulate breathing behavior in the spring. I previously found that a well-studied homeostatic mechanism called synaptic scaling increases the strength of excitatory synapses in inactive motor neurons and helps to maintain the respiratory motor outflow after 3 months without breathing (Santin et al., 2017, *eLife*). Given that intrinsic changes are also known to counterbalance reduced activity, I tested the hypothesis that neuronal excitability is enhanced following months of respiratory motor inactivity. To test this hypothesis I measured several intrinsic electrophysiological properties from 96 respiratory motor neurons (68-control and 28-3 months of inactivity). Hierarchical clustering determined that there are two types of neurons in this nucleus with either "fast" or "slow" firing properties. These neurons can be separated based on their maximum firing rate and input resistance. In "fast" firing neurons I found that the current required to elicit an action potential (rheobase) was reduced in response to 3 months without activity ($p=0.006$). In "slow" firing neurons the rheobase was the same between the two groups, but the frequency-current (F-I) relationship was steeper after inactivity ($p=0.04$). Thus, intrinsic excitability is enhanced after months of natural inactivity. Collectively, these results suggest that multiple homeostatic forms of plasticity may be an adaptation in neural systems that are exposed to dramatic activity challenges as a part of the animal's life history. A better of the understanding of the relationship

between neuronal homeostasis and behavior may be found by studying animals that inhabit extreme environments.

Disclosures: J.M. Santin: None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.17/P22

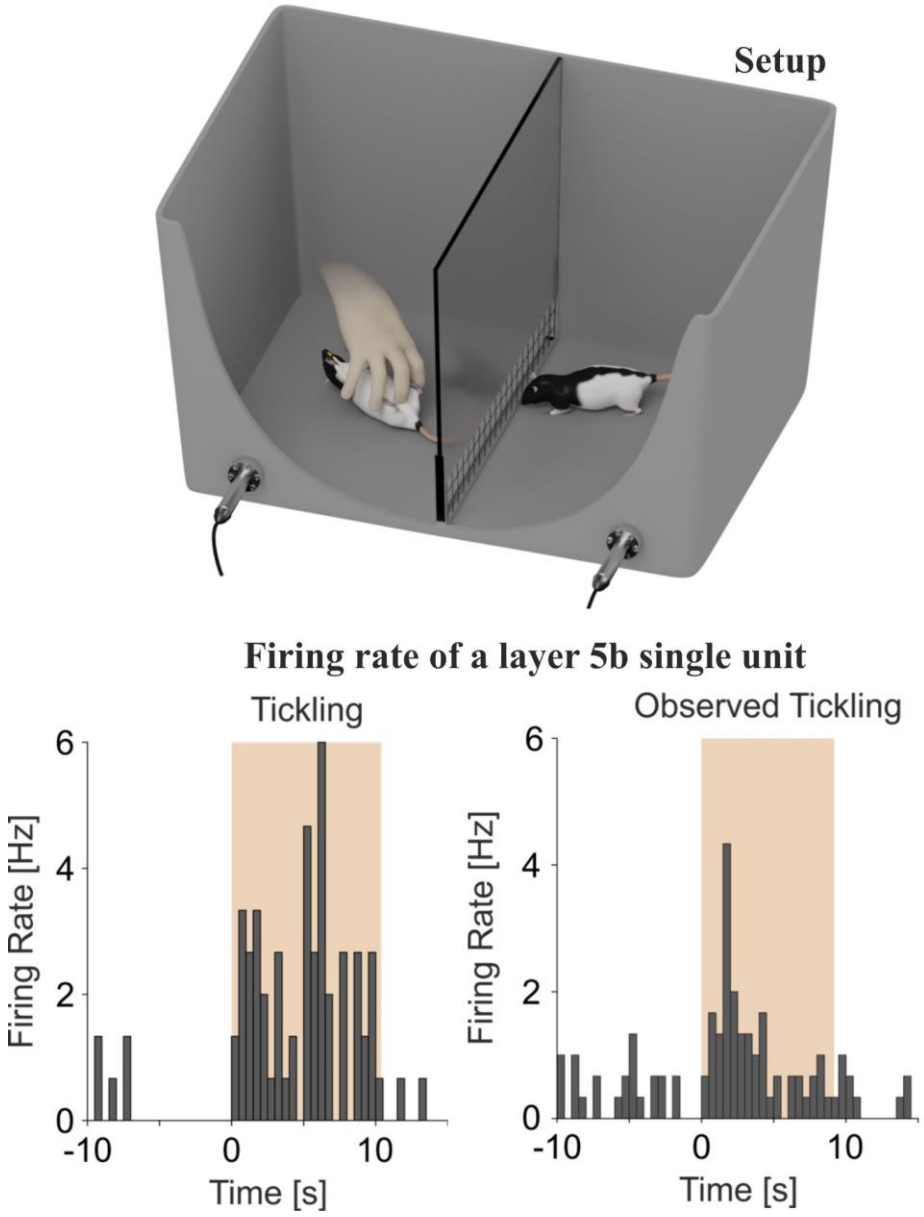
Topic: F.01. Neuroethology

Support: DFG Grant 393810148
DFG Leibnitz Prize
NeuroCure Cluster of Excellence

Title: Cortical and behavioral contagion of ticklishness in rats

Authors: *L. KAUFMANN[1], M. BRECHT[1], S. ISHIYAMA[2];
[1]Humboldt University/BCCN Berlin, Berlin, Germany; [2]Pathophysiology, Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Mainz, Germany

Abstract: Laughter is highly contagious in humans, and empathy for positive emotions plays a critical role in social interactions. It has been shown previously, that a basal form of empathy (Panksepp & Panksepp 2013) can be seen in rodents. Emphatic behavior in rodents has mostly been studied in the context of negative emotions, i.e. pain or stress (Panksepp & Lahvis 2011). Earlier studies showed, that rats respond to being tickled by humans with ultrasonic vocalizations resembling laughter, and ticklishness is represented in deep layers of the somatosensory cortex (Ishiyama & Brecht 2016). It remains unknown, however, whether ticklishness and "laughter" are contagious in rats. Thus, our research aims to clarify, whether ticklishness and its associated playful emotions are contagious in rats, and, if this is the case, what the neuronal representations of observed ticklishness might be.
To this end, we recorded neuronal activity in the trunk somatosensory cortex in an "observer rat" while it is tickled, receiving audio/visual/audiovisual playback of rat tickling footage, and while it is observing another rat, the "demonstrator rat", being tickled.
As found previously, the rats showed a neuronal and behavioral response to tickling. The observer rat seemed to pay attention to the demonstrator rat being tickled, and occasionally showed "Freudensprünge" (joy jumps). Some trunk somatosensory neurons responded not only to tickling, but also to observed tickling. Analysis is still ongoing, but it seems like there is indeed a small fraction of neurons showing these mirror-like effects. These results suggest ticklishness is contagious and observed ticklishness is represented in the trunk somatosensory cortex in rats.



Ishiyama S., Brecht M. (2016). Neural correlates of ticklishness in the rat somatosensory cortex. *Science*, 354(6313), 757-760.
Panksepp J., Lahvis G. (2011). Rodent empathy and affective neuroscience. *Neuroscience & Biobehavioral Reviews*, 35(9), 1864-1875.
Panksepp J., Panksepp J. (2013). Toward a cross-species understanding of empathy. *Trends in neurosciences*, 36(8), 489-496.

Disclosures: L. Kaufmann: None. **M. Brecht:** None. **S. Ishiyama:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 068.18/P23

Topic: F.01. Neuroethology

Support: HHMI
NIMH

Title: Functional properties of a thalamocortical circuit for the control of social behavior

Authors: *A. C. NELSON[1], V. KAPOOR[1], J. A. GNANASEGARAM[1], E. VAUGHN[1], V. N. MURTHY[1], C. G. DULAC[2];
[1]Mol. and Cell. Biology; Ctr. for Brain Sci., [2]Mol. and Cell. Biology; Ctr. for Brain Science; HHMI, Harvard Univ., Cambridge, MA

Abstract: Social hierarchy is an organizing principal for animal society. A long-standing question has concerned how functional circuits at the molecular and cellular level diverge as social rank emerges. Here, we uncover the characteristics of social rank at the level of gene expression, cell types, neuronal plasticity, and behavior. A paradigm to capture the emergence of hierarchy identifies the mediodorsal thalamus (MDT) and the caudal anterior cingulate cortex (cACC) as two interconnected brain areas showing differential activation between dominant and subordinate mice. MDT is required for hierarchy formation, and MDT glutamatergic neurons and their recipient GABAergic partners in the cACC are sufficient to control competitive performance. We identify persistent, rank-dependent changes in the synaptic inputs and biophysical properties of MDT neurons. Single cell analysis reveals a link between rank and transcription of voltage gated ion channels, including a cation channel that potentiates excitatory transmission in the MDT-cACC circuit. We propose a model where synaptic plasticity in the MDT instructs feedforward inhibition of the cACC to control expression of social avoidance behavior during social competition.

Disclosures: A.C. Nelson: None. **V. Kapoor:** None. **J.A. Gnanasegaram:** None. **E. Vaughn:** None. **V.N. Murthy:** None. **C.G. Dulac:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: F.01. Neuroethology

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Ministry of Science and Technology Office China 973 program 2015CB559201
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the Strategic Priority Research Program of the Chinese Academy of Sciences XDBS01010200

Title: Reciprocal antagonism between hunger and maternal care

Authors: *X. XU, X.-Y. LI, Y. HAN, W. ZHANG;
Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China

Abstract: AGRP (agouti-related neuropeptide) expressing inhibitory neurons sense caloric needs of an animal to coordinate homeostatic feeding. Recent evidence suggests that AGRP neurons also suppress competing actions and motivations to mediate adaptive behavioral selection during starvation. Previously, our lab shows that presence of pups suppresses hunger or AGRP neuronal activation induced feeding. Here, we show that AGRP neurons form inhibitory synapses onto ~30% neurons in the medial preoptic area (mPOA), a region critical for maternal care. Remarkably, optogenetically stimulating AGRP neurons decreases maternal nest-building while minimally affecting pup retrieval, partly recapitulating suppression of maternal behaviors during food restriction. In parallel, optogenetically stimulating AGRP projections to the mPOA or to the paraventricular nucleus of hypothalamus but not to the LHA (lateral hypothalamus area) similarly decreases maternal nest-building. Chemogenetic inhibition of mPOA neurons that express Vgat (vesicular GABA transporter), the population targeted by AGRP terminals, also decreases maternal nest-building. In comparison, chemogenetic inhibition of neurons in the LHA that express vesicular glutamate transporter 2, another hypothalamic neuronal population critical for feeding and innate drives, is ineffective. Importantly, nest-building during low temperature thermal challenge is not affected by optogenetic stimulation of AGRP→mPOAprojections. Finally, via optogenetic activation and inhibition we show that distinctive subsets of mPOA Vgat+ neurons likely underlie pup retrieval and maternal nest-building. Together, these results show that AGRP neurons can modulate maternal nest-building, in part through direct projections to the mPOA. This study corroborates other recent discoveries and underscores the broad

functions that AGRP neurons play in antagonizing rivalry motivations to modulate behavioral outputs during hunger.

Disclosures: **X. Xu:** None. **X. Li:** None. **Y. Han:** None. **W. Zhang:** None.

Poster

068. Vertebrate Sensory-Motor Integration

Location: Hall A

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Topic: F.01. Neuroethology

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National Natural Science Foundation of China/81870891
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Fund for Jiangsu Province Specially-Appointed Professor/2018
Natural Science Foundation of Jiangsu Province/ BK20171160
The Natural Science Foundation of the Jiangsu Higher Education Institutions of China/17KJA320007
The Natural Science Foundation of the Jiangsu Higher Education Institutions of China/18KJA320009

Title: Subthalamic glutamatergic neurons encode magnitude and valence of food and state-dependently regulate food intake in mice

Authors: *C. XIAO, C. ZHOU, H. WU, X. YAN, W. GU, Y. LUAN;
Xuzhou Med. Univ., Xuzhou, China

Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an effective therapy for motor deficits in Parkinson's disease (PD), but incurs weight gain in most late-phase PD patients. It is unknown whether the STN responds to consumption of solid food and regulates food intake. To elucidate these questions, we applied fiber photometry and optogenetic techniques to respectively monitor and regulate the activity of STN glutamatergic neurons in freely moving mice. STN neurons augmented activity in response to food consumption with discrimination of both the magnitude (larger vs smaller food pallets) and valence (regular vs bitter food) of the food. The responses diminished upon satiation, when the frequencies of both spontaneous and depolarizing stimulation-evoked firings of STN neurons were significantly suppressed. In sated state, STN neurons displayed greater responses to palatable food than to regular food. Interestingly, optogenetic stimulation and inhibition of STN neurons did not change short-term food intake in hungry and sated mice in the light cycle, but respectively reduced and enhanced food intake in the dark cycle without fasting. The increase of food intake following the inhibition of the STN might be related to its rewarding effects because pairing STN photo-

inhibition with the entrance of the freely moving mice into a distinct place established conditioned place preference. Our results support that STN neurons are implicated in the consumption of solid food and regulate food intake.

Disclosures: C. Xiao: None. **C. Zhou:** None. **H. Wu:** None. **X. Yan:** None. **W. Gu:** None. **Y. Luan:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.01/P26

Topic: F.02. Behavioral Neuroendocrinology

Support: CONACyT 252756, 253631
INPER 2018-1-63
UNAM-DGAPA-PAPIIT IN202818, IN203518
INPER 212250-3230-21216-05-15
NIH P51OD11132

Title: Cohabitation with mating induces place preferences in biparentally reared, but not monoparentally reared male prairie voles

Authors: *G. VALERA-MARIN[1], F. CAMACHO[1], N. F. DÍAZ[2], L. J. YOUNG[3], R. G. PAREDES[1], W. PORTILLO[1];
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Abstract: Social monogamy and the formation of long-lasting pair bonds is characteristic of humans and many other primates. In mammals, around 3-9% use this reproductive strategy which is commonly associated with the biparental care of the offspring. *Microtus ochrogaster*, or prairie voles, are socially monogamous rodents who live in small familiar groups where the mother and the father take care of their offspring. Voles raised in monoparental families, by their mother only, require up to 1 week of cohabitation with a partner to establish a pair bond, while those raised in biparental families usually need less than 24 hours. During cohabitation, mating is important to establish a pair bond; if during cohabitation either mating or the reward state induced by mating are prevented, they won´t build a pair bond. We hypothesized that a difference in any of these aspects of mating may explain the delay in pair bond formation in monoparental voles. In the present experiment we evaluated both mating and its associated reward state in mono and biparentally reared voles. Regarding mating, no significant differences were found between mono and biparental males. The reinforcing properties of mating and

cohabitation were evaluated using the conditioned place preference test. Preliminary data show that biparental males increase the time in the mating-associated chamber ($p<0.05$) but monoparental males do not. Thus, monoparental raised males mated as the biparental males did, but this behavior did not result in a change in place preference, suggesting attenuated mating induced reward. This result may explain the delay in the pair bond formation in monoparental male prairie voles.

Disclosures: G. Valera-Marin: None. **F. Camacho:** None. **N.F. Díaz:** None. **L.J. Young:** None. **R.G. Paredes:** None. **W. Portillo:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.02/P27

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant 1R15HD075222-01A1

Title: The influence of tyrosine hydroxylase neurons on pair-bonding behaviors of a socially monogamous rodent

Authors: *J. B. LICHTER[1], M. S. MCMURRAY[2], B. KEANE[3], N. G. SOLOMON[1]; [1]Biol., [2]Psychology, Miami Univ., Oxford, OH; [3]Biol. Sci., Miami Univ., Hamilton, OH

Abstract: The degree of social monogamy and pair-bonding, as observed in birds and mammals, is influenced by neurophysiological pathways, such as the dopamine system. In prairie voles (*Microtus ochrogaster*), characterized as a socially monogamous rodent species, the dopamine and norepinephrine producing pathways can be studied using tyrosine hydroxylase (TH), a rate limiting factor in the production of dopamine. The posterior bed nucleus of the stria terminalis (BNST) and the medial amygdala (MeA) contain populations of TH positive neurons, which are absent in polygamous meadow voles (*M. pennsylvanicus*). This difference suggests that TH neurons in these brain areas might be present or differentially activated only in males that are pair-bonded. We tested whether TH positive neurons within the pair-bonding neural network accurately predicted pair bonding in male prairie voles. We obtained 22 paired males and 25 unpaired male prairie voles from our semi-natural populations to examine differences in TH within the BNST, MeA and ventral tegmental area. Results will be discussed.

Disclosures: J.B. Lichter: None. **M.S. McMurray:** None. **B. Keane:** None. **N.G. Solomon:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.03/P28

Topic: F.02. Behavioral Neuroendocrinology

Support: CONACYT 252756, 253631; UNAM-DGAPA-PAPIIT IN202818, IN203518

Title: Kinship, mating and social preference in female prairie voles microtus ochrogaster tested in a multiple partner paradigm

Authors: *A. FERREIRA-NUÑO[1], F. CAMACHO[2], N. DÍAZ-MARTÍNEZ[3], L. J. YOUNG[4], R. PAREDES-GUERRERO[2], W. PORTILLO-MARTINEZ[2];
[1]Biología de la Reproducción, Univ. Autonoma Metropolitana, México, Mexico; [2]Inst. de Neurobiología,, Univ. Nacional Autónoma de México, Querétaro, México, Mexico; [3]Inst. de Perinatología., México, Mexico; [4]Ctr. for Translational Social Neurosci., Emory Univ., Atlanta, GA

Abstract: The prairie vole (*Microtus ochrogaster)* is one of the few species of mammals with a socially monogamous reproductive strategy. Following 6 h of cohabitation with mating, prairie voles establish enduring pair bonds characterized by partner preference and selective huddling with the partner. Here we use a novel multiple partner preference paradigm to examine the potential effect of kinship on social preferences, mate choice and selective huddling in female prairie voles. The multiple partner paradigm is made with 4 Plexiglas cylinders arranged in a cross configuration (Fig. 1). Each cylinder has a hole leading to a central neutral compartment allowing the females to freely enter each cylinder housing a different stimulus male. Each female (N=9) was tested individually with 4 tethered stimulus males, the father, a male sibling, a cousin and an unrelated male. Except for the father, all animals were sexually naïve. Subject females were primed with estradiol benzoate (0.5 µg / vole) for 4 consecutive days to induce sexual receptivity and were introduced into the central compartment allowing them to interact with the 4 stimulus males for 6 h. The complete test was recorded on video and mating and time spent huddling with each male was recorded. The preferred male was the male with whom the female spend more time throughout the test. Four out of 9 females preferred the cousins, 2 the father, 2 the unrelated male and only 1 choose the sibling. Three females mated with 2 stimuli males during the test, 4 only with one male and 2 did not mate with anyone. All mating females showed huddling behavior exclusively with the preferred male and this always was the one they mated with. Our results suggest that familial kinship does not strongly bias affiliation preference or mate choice in this paradigm in female prairie vole and that huddling is a good indicator partner preference. The multiple partner paradigm should be useful for examining mate choice and partner preference development in prairie voles.

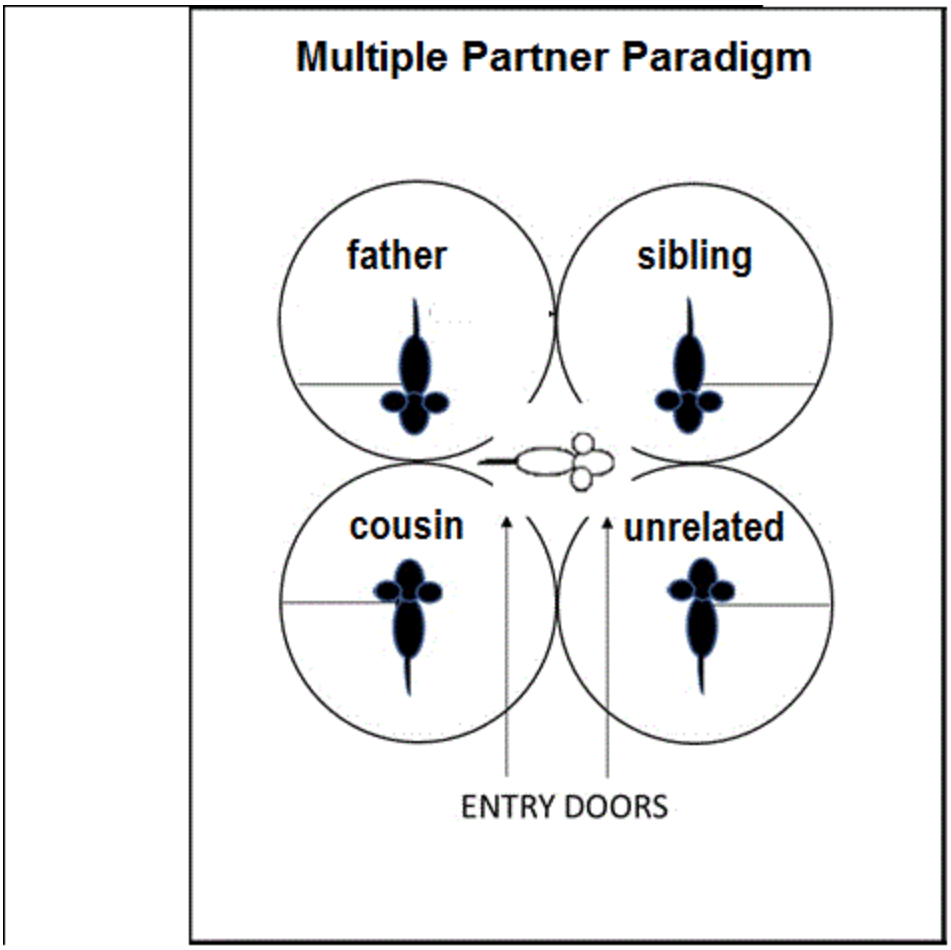

FIG. 1. THE MULTIPLE PARTNER PARADIGM (MPP) WAS MADE WITH 4 TRANSPARENT ACRYLIC CYLINDERS ARRANGED IN A CROSS FASHION WITH THEIR WALLS JOINED. EACH CYLINDER HAS 45 CM IN DIAMETER, 20 CM IN HEIGHT AND A HOLE (4 X 4 CM) ON THE FLOOR, ALLOWING THE RECEPTIVE FEMALE TO GO BACK AND FORTH FROM EACH CYLINDER TO THE CENTRAL SECURITY CHAMBER NATURALLY FORMED BY THE ALIGNMENT OF THE 4 CYLINDERS. FEMALE MATED FOR 6 HR WITH 4 MALES: THE FATHER, THE SIBLING, THE COUSIN AND UNRELATED MALE, (ONE CHAMBER BY MALE).

Disclosures: A. Ferreira-Nuño: None. **F. Camacho:** None. **N. Díaz-Martínez:** None. **L.J. Young:** None. **R. Paredes-Guerrero:** None. **W. Portillo-Martinez:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.04/P29

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1455960
American Ornithological Society Van Tyne Award
Start-up funds to R.M.Calisi

Title: The role of prolactin in maintaining the parental brain

Authors: *V. S. FARRAR, R. C. VIERNES, R. M. CALISI;
Neurobiology, Physiol. and Behavior, Univ. of California, Davis, Davis, CA

Abstract: While parenting offspring, many animals, including humans, experience reduced sexual behavior and fertility trade-offs to their own survival and future reproductive opportunities. We tested the role of prolactin, a hormone that facilitates parental care across vertebrates, in mediating such trade-offs. As in mammals, prolactin drives various avian parental care behaviors, including the phenomenon of lactation in both mothers *and* fathers of the biparental rock dove species (*Columba livia*). This particular trait in rock doves provides a rare opportunity to control for potential sex-biased confounds surrounding prolactin-mediated lactation. To test the effects of prolactin on the maintenance of the maternal versus paternal brain in the absence of offspring, we experimentally manipulated offspring presence and prolactin levels. As compared to controls, we identified significant differential gene transcription in the hypothalamic-pituitary-gonadal (HPG) axis, a biological system whose function is essential for the expression of reproductive behaviors in vertebrates. We present the causal effects of elevated prolactin on the maintenance of a HPG gene transcription profile characteristic of the maternal and paternal brain.

Disclosures: V.S. Farrar: None. **R.C. Viernes:** None. **R.M. Calisi:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.05/P30

Topic: F.02. Behavioral Neuroendocrinology

Title: Gestational exposure to a ketogenic diet improves sociability and affect in CD-1 mice

Authors: K. G. FLYNN, A. M. S. ARQOUB, ***L. A. MARTINEZ***;
Neurosci., Trinity Col., Hartford, CT

Abstract: High-fat, low-carbohydrate ketogenic diets (KDs) are viable treatment options for refractory epilepsy when administered postnatally; however, their impact on gestational development remains unclear. Previous studies have determined that gestational KD exposure (GKD) improves affect and decreases anxiety in CD-1 mice. Furthermore, postnatal exposure to a KD has been found to increase sociability across a range of rodent models of autism. Here we examined how sociability is impacted by GKD. Given that the neuropeptide oxytocin (OT) positively regulates affect, anxiety, and sociability, we also examined the effects of GKD on OT expression. In this study, CD-1 mice were gestationally exposed to either a control diet or a KD and then tested as adults for sociability (three chambered apparatus) and affect (forced swim test). Brain tissue was then immunohistochemically processed for OT expression in the paraventricular nucleus of the hypothalamus (PVH) and the bed nucleus of the stria terminalis (BNST). We found that GKD increased sociability and reduced depressive-like symptoms without significantly impacting OT expression in the PVH or BNST. These results indicate that GKDs may have novel therapeutic applications for developmental disorders that feature social deficits.

Disclosures: K.G. Flynn: None. **A.M.S. Arqoub:** None. **L.A. Martinez:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.06/P31

Topic: F.02. Behavioral Neuroendocrinology

Support: DFG Grant: BR 3479/11-1

Title: Neural mechanisms of kinship behavior in the rat

Authors: *A. CLEMENS, M. BRECHT;
Humboldt-Universität zu Berlin, Berlin, Germany

Abstract: According to Hamilton’s inclusive fitness hypothesis, kinship is an organizing principle of social behavior. There is abundant behavioral evidence supporting the predictions of this hypothesis, including the ability to recognize kin and the adjustment of behavior based on kin preference with respect to altruism, attachment and care for offspring in insect societies. Despite the fundamental importance of kinship behavior, the underlying neural mechanisms are poorly understood. We repeated behavioral experiments originally performed by Hepper to test

the recognition ability and behavioral preference of rats for their kin. Consistent with Hepper's work, we find a developmental time course for kinship behavior, where rats prefer interactions with their siblings at young ages and express non-sibling preferences at older ages. In probing the brain areas responsible for this behavior, we find that aspiration lesions of the lateral septum but not control lesions of cingulate cortices eliminate the behavioral preference in young animals for their siblings and in older rats for non-siblings. We then performed in vivo juxta-cellular and whole cell patch-clamp recordings in the lateral septum of awake and anaesthetized rats and find neuronal responses to social stimuli. In particular, we observed responses to multisensory kin stimuli in the intermediate lateral septum. In ongoing experiments, we are examining the molecular and cellular identity of kin-responsive neurons in the lateral septum. The intermediate lateral septum receives massive inputs from ventral hippocampus and outputs to a variety of subcortical targets relevant for social behaviors. Thus, our lesion, physiology and anatomy results are consistent with a role of the lateral septum in organizing mammalian kinship behavior.

Disclosures: A. Clemens: None. **M. Brecht:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.07/P32

Topic: F.02. Behavioral Neuroendocrinology

Title: Effects of a FAAH inhibitor on sociability and social novelty preference in adolescent male and female rats

Authors: C. JOHNSTON[1], Z. R. MICHAS[1], H. STADLER[1], O. LYONS-POTTER[1], K. HICKS[1], R. WITMER[1], ***H. H. LOPEZ**[2];
[1]Neurosci. Program, [2]Psychology Dept., Skidmore Col., Saratoga Springs, NY

Abstract: The endocannabinoid (eCB) system contributes substantially to the development and expression of social behavior in mammals. Enhancement of eCB activity can boost social play in juvenile rats and rescue social preference deficits in various rodent models of autism. However, few laboratories have directly compared the pro-social effects of eCB agonists in males and females. Furthermore, the effect of eCB agonism on social memory has not been explicitly tested. In the present experiment, we manipulated anandamide (AEA) signaling via systemic administration of URB597, a fatty acid amide hydrolase (FAAH) inhibitor. 72 adolescent male and female Wistar rats were given a single intraperitoneal injection of either vehicle, 0.1 mg/kg URB597, or 0.3 mg/kg URB597 60 minutes prior to behavioral testing. A three-chambered apparatus was utilized to sequentially test social preference and social novelty preference. During each of these assays, entries into each chamber, time spent in each chamber, and

investigation time of targets were recorded. We hypothesized that URB597 treatment would dose-dependently increase sociability (preference for a social target vs. an empty cage) but decrease social novelty preference (preference for a novel conspecific vs. a familiar conspecific). While all groups showed a significant social preference and social novelty preference, there were no significant effects of URB597 on either sociability or social novelty preference. There were no significant interactions between drug treatment and sex of subject. There was a main effect of sex on sociability, such that males expressed greater social preference overall compared to females. There was also a main effect of drug on chamber entries in female subjects in the social novelty preference task, such that vehicle-treated subjects made more chamber entries than subjects in the drug-treatment groups. We suggest that boosting AEA activity may enhance social behavior in animals with pre-existing deficits (e.g., in animal models of autism), but not in normally developing adolescent rats.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.08/P33

Topic: F.02. Behavioral Neuroendocrinology

Support: Project Lisboa-01-0145-FEDER-030627

Title: Perceptual mechanisms of social affiliation and the role of oxytocin in zebrafish

Authors: *A. S. NUNES[1], L. CARREIRA[1], S. ANBALAGAN[2], J. BLECHMAN[2], G. LEVKOWITZ[2], R. OLIVEIRA[1];
[1]Inst. Gulbenkian de Ciência, Lisboa, Portugal; [2]Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Visual recognition of individuals is crucial for social interactions and survival. Socially relevant visual information is extracted from the environment and processed in specific brain centers to guide social behaviors. However, little is still known about how visual cues contribute to social affiliation. In this work we considered two main social visual features in zebrafish: conspecific form and biological motion and used videoplaybacks to address their specific contribution on social affiliation. We demonstrated that each cue is sufficient to promote social attraction, and the combination of the two induces a robust preference towards a conspecific. The regulation of this visual processing mechanism is mediated by oxytocin, a neuromodulator of social behaviors across species, regulating conspecific form and motion differently. These findings support the hypothesis that the ubiquitous effects of oxytocin on

different aspects of social behavior across species can be due to its action on very basic perceptual mechanisms underlying the recognition of conspecifics.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.09/P34

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF Award 1149446

Title: Hippocampal neuropeptide-Y in relation to social behavior in the African naked mole-rat

Authors: *C. A. D. DUNNE-JAFFE[1], D. P. MCCLOSKEY[2];
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Abstract: The African Naked Mole-Rat (Heterocephalus Glaber, NM-R) is a fossorial rodent with a eusocial cooperative breeding organization. This species adapts well to a laboratory housing environment and continues to demonstrate innate, ethologically relevant colony maintenance behaviors even after generations of captive breeding. We have previously identified a division of labor among colony workers, using RFID technology, with larger animals participating more in colony maintenance behaviors in and around the nest chamber (defense, nest building, tunnel excavation) and smaller animals participating more in digging and foraging behaviors distal to the nest (in a sand digging task). Here, we measured whether neuropeptide Y (NPY), an important biomarker for caste differences in eusocial insects, also varies with caste in the hippocampus of NM-R. Animals from two captive naked mole-rat colonies were tracked for their participation in colony maintenance tasks over a 10-day period. Animals were selected based on performance of these tasks, and hippocampal NPY expression was evaluated with confocal microscopy of immunohistochemically-labeled tissue and measured quantitatively using ELISA. In general, animals showed an unusual pattern of expression, with NPY staining restricted to what appear to be axonal filaments in the stratum radiatum region of CA1. Quantitative analysis of NPY expression revealed a trend toward significance, with larger nest-centric animals more likely to show elevated hippocampal NPY.

Disclosures: C.A.D. Dunne-Jaffe: None. **D.P. McCloskey:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.10/P35

Topic: F.02. Behavioral Neuroendocrinology

Title: Social decision-making network activation and behavior following pair reunion and in response to an infidelity challenge in monogamous zebra finches

Authors: A. M. SMITH[1], K. K. BOEDIGHEIMER[1], H. HAWES[1], K. G. SWANSON[1], I. D. PHAM[1], *S. A. HEIMOVICS[2];
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Abstract: The neurobiology of social monogamy has intrigued scientists and been the focus of neuroscience research for decades. Pair-bonding and partner preference are key features of monogamous mating systems yet extrapair copulation and extrapair paternity are relatively common among socially monogamous species. Despite many exciting advances in our understanding of the behavioral neuroendocrinology of social monogamy, remarkably little is known about how the brain maintains pair bonds and regulates behavior when those bonds are challenged. In order to address this gap in our understanding of the neurobiology of social monogamy, this research examines activity in the social decision-making network (SDMN) of male and female zebra finches (Taeniopygia guttata) separated from their pair-bonded mate overnight and subsequently presented with either their pair-bonded mate or a novel opposite-sex conspecific (referred to as an “infidelity challenge” hereafter). A preliminary study in our lab revealed that 71.4% (5 out of 7) of pair-bonded males presented with infidelity challenge engaged in extrapair copulation standing in stark contrast to the 12.5% (1 out of 8) of pair-bonded males that copulated when presented with their mate. Current experiments are ongoing to examine potential sex differences in behavioral response to infidelity challenge as well as immediate early gene expression in the SDMN of subjects. Specifically, brains will be collected from male and female subjects after a 30 min infidelity challenge (or mate presentation control) and SDMN nuclei will be dissected using the Palkovits punch technique. Truck blood will also be collected at the time of sacrifice in order to quantify circulating levels of steroid hormones in subjects. rt-qPCR will be used to quantify relative levels of c-fos and ZENK mRNA in punches in order to gain insight into SDMN-wide activation associated with differences in subject responses to behavioral testing. Given that the literature frequently describes zebra finches as a genetically monogamous species, our findings will provide novel insight into the SDMN nodes that appear to central to maintaining exclusive pair bonds and/or regulating behavior when those bonds are broken.

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Poster

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Program #/Poster #: 069.11/P36

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF DGE 1106400

Title: Same-sex affiliation in female meadow voles: The role of environmental conditions and nonapeptides

Authors: *N. ONDRASEK[1], S. M. FREEMAN[3], M. PALUMBO[2], I. ORELLANA BONILLA[1], E. SALDANA[1], R. M. CALISI[1], K. L. BALES[2], I. ZUCKER[4];
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Abstract: In female meadow voles, both environmental conditions and the nonapeptide systems—including the oxytocin and vasopressin systems—influence same-sex affiliation, yet we know little about how these factors interactively mediate social behavior. To examine how winter-typical environmental conditions influence nonapeptide-mediation of same-sex affiliation, we housed paired females in varying combinations of day length, temperature, and food availability for 7 weeks, tested them for social preference, and then examined the density and distribution of central oxytocin and vasopressin 1a receptors using autoradiography. Oxytocin receptor (OTR) density was pronounced in several brain regions, including the lateral septum (LS), central amygdala, prefrontal cortex (PFC), and the core and shell regions of the nucleus accumbens (NAcc core and NAcc shell, respectively). Vasopressin 1a receptor (V1aR) density was pronounced in the LS, paratenial thalamus (PT), ventral hippocampus, and ventral pallidum (VP). Day length impacted OTR and V1aR densities, such that short day (SD) voles had greater V1aR density in the LS, and long day (LD) voles had greater OTR density in the NAcc shell when exposed to low temperatures. Huddling behaviors were correlated with binding densities in several brain areas, including the PT, NAcc core, VP, and PFC. In several cases, the correlation between behavior and receptor density was only apparent under certain environmental conditions. Time spent huddling with a novel individual was negatively correlated with V1aR density in the PT and VP, though the latter was only apparent in moderate temperatures in SDs. In SDs, huddling with a partner was positively correlated with V1aR density in the PT; under food restriction, huddling with a partner was negatively correlated with OTR binding in the NAcc core. Our results suggest that in meadow voles, environmental conditions modulate these

nonapeptide systems, and that the manner in which these systems mediate same-sex affiliation varies with changes in day length, temperature, and food availability. Our findings also suggest a previously unknown role for the PT—which projects to the limbic system and plays a critical role in affective behaviors such as feeding, stress, and anxiety—in mediating same-sex social affiliation.

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Poster

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant P01 HD07575

Title: Perinatal oxytocin transforms sex differences in brain structure and function in prairie voles: An MRI study

Authors: *J. R. YEE[1], C. CARTER[2], X. CAI[3], W. KENKEL[4], A. M. PERKEYBILE[5], P. P. KULKARNI[6], C. F. FERRIS[7];
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Abstract: The existence of sex differences in the brain have become impossible to ignore. Research in non-human animals provides an opportunity to study the biology of sex differences in isolation from the roles that culture and society play in shaping the brain in humans. Furthermore, it allows controlled investigations into factors hypothesized to critically alter the emergence of sex differences in the brain. One such factor, the neuropeptide oxytocin (OT), is intricately involved in the mammalian birth process and synthetic OT (Pitocin) is widely used to induce or augment labor. Thus, the aim of this study was to characterize neural structure and function in male and female prairie voles (*Microtus ochrogaster*), an excellent model of labor induction and parental behavior because they exhibit social monogamy and high levels of biparental care of pups. In addition to male vs female comparisons, we also investigated the impact of perinatal oxytocin administration, a common obstetric practice that we have recently shown to induce changes in social behavior, DNA methylation and gene expression following various experiences including differential parenting and exposure to neuropeptides. To examine

functional, microstructural and macrostructural differences in the brain we employed a multimodal magnetic resonance imaging (MRI) approach. Voles were examined for changes in functional coupling across integrated neural circuits using resting state blood oxygen level dependent (BOLD) functional connectivity, alterations in gray matter microarchitecture using diffusion-weighted imaging with quantitative anisotropy and changes in regional volumes using voxel-based morphometry. MRI scans were performed approximately 3 months post-exposure. All images for each modality were registered to a 3D MRI Vole Atlas with 117 segmented and annotated brain areas used to generate an unbiased computational analysis of all data. Results demonstrate profound changes as a function of both sex and perinatal oxytocin administration. While conventionally-reared voles showed minimal difference in regional brain volumes between the sexes, they demonstrated widespread differences in gray matter microstructure. In contrast, oxytocin-exposed voles showed widespread differences in regional brain volumes between the sexes, but minimal difference in gray matter microstructure. In addition to these structural changes, the effect of sex and oxytocin-administration on resting state functional connectivity will be presented. This is among the first studies to demonstrate the impact of sex and a common biomedical procedure on structure and function of the brain.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Program #/Poster #: 069.13/P38

Topic: F.02. Behavioral Neuroendocrinology

Support: JSPS KAKENHI Grant Number JP17H01888

Title: Suitability of sociality research using Hatano rats

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Abstract: Expression of appropriate social behavior is important for animals that live in groups, but there are many unclear aspects. Although autism model rodents are often used in social tests, this model is not suitable studying differences in sociality of normal individuals. Inbred animals are one of the good model to study this, however, inbred rats with well characterized sociality had not been established. We have studied Hatano rats (High active avoidance rats; HAA, Low active avoidance rats; LAA), inbred strains selected from Sprague-Dawley rats (SD) by active

avoidance test, and found that there is a difference between them in social investigation test. This result suggests that HAA and LAA may have different sociability characteristics. Consequently, studies with Hatano rats may provide a new perspective on sociability research. Therefore, in this study, we conducted social behavior test to examine whether Hatano rats could be a useful model for sociality research.We group-bred for each strain (HAA, LAA, SD) from 3 weeks of age, and conducted the behavior test at 5 to 7 weeks of age. Detail of this test is as follows: the male rat was placed in a 29 × 70 × 30 cm box with a tube (Blank) for 3 minutes, then the male rat which had reared in the same cages (Familiar) or in different cages (Unfamiliar) was placed in a tube for 3 minutes each. The approach time to other individuals (tube) in the 3 minutes was measured and used as an evaluation index of sociality acquisition. At 5 weeks of age, there was no significant difference in access time to the Familiar and Unfamiliar in all strains. However, at 6 and 7 weeks of age, the access time to Familiar was significantly longer than that of Unfamiliar in LAA and SD, but no significant difference was observed in HAA.In this experiment, there was a clear strain difference in the approach to Familiar and Unfamiliar. Thus, it is likely that Hatano rats could be a useful model for sociality studies. We conducted a play behavior that as part of social behavior research using Hatano rats. In addition, we quantitatively measured the level of oxytocin mRNA expression related to the sociality in the amygdala to reveal the mechanism of the difference in observed behavior. These results are currently under analysis and will be discussed. This work was partly supported by JSPS KAKENHI Grant Number JP17H01888.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant P51 OD011133

Title: Analysis of hair cortisol concentration in captive chimpanzees (pan troglodytes): Correlates with group size and dynamics

Authors: *K. A. PHILLIPS[1,2], A. BEARMAN[1], K. JASPE[1], K. M. BRASKY[2], M. A. DE LA GARZA[2];
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Abstract: Measuring cortisol in hair has become an increasingly popular method for evaluating relative concentrations of cortisol as it is a non-invasive technique that represents a cumulative

measure of long-term hypothalamic-pituitary-adrenal (HPA) axis activation. This measure is proposed to assess long-term stress. Management of captive chimpanzees requires periodic reconfiguration of the social group in response to changing social dynamics, health considerations, and other issues. We investigated hair cortisol concentration in captive chimpanzees in order to evaluate the effects of health, social stability and social grouping. Hair was collected from 58 chimpanzees (29 males, mean age 24.8 years; 29 females, mean age = 28.1 years) for whom detailed data was available on health, group size, and group stability. Hair samples were opportunistically collected from the chest region and 0.5 cm of the hair sample closest to the skin (representing cortisol from the past month) was analyzed via methanol extraction and enzyme immunoassay. Factors known to influence results, namely fineness of grinding and extraction time, were kept constant across all samples. We expect hair cortisol concentration to show correlations with group size and unstable group dynamics. These results will be discussed in the context of understanding how stability of social relationships and health influence long-term HPA axis activity.

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Poster

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Topic: F.02. Behavioral Neuroendocrinology

Support: PTDC/BIA-ANM/0810/2014

Title: The role of developmental social complexity on adult zebrafish social behaviour

Authors: *M. C. TELES[1], C. GONÇALVES[1], R. F. OLIVEIRA[1,2];
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Abstract: The social brain hypothesis (SBN) posits that cognitive and brain evolution is mainly driven by the social environment. Although this relationship between social context and cognitive abilities has been shown in primates at the evolutionary scale (group size as a measure of complexity, and relative brain size of the neocortex as an indicator of cognitive skills), violations of this hypothesis have been reported when trying to generalize it across vertebrate taxa. So far, this hypothesis has ignored the potential effects of developmental processes, which may help to explain some of the current controversies, since early experience is essential in the development of the adult phenotype. In the present work, we studied the proximate causes of brain evolution: how development affects cognitive functions and neuronal numbers. Therefore, zebrafish were raised in different social environmental complexities (i.e. group size and group

stability) until adulthood and tested for their social abilities, and neuronal numbers in different brain regions. At the behavioural level, our results indicate differences between the groups, for instance, group cohesion is influenced by group size, while shoal preference is influenced by group stability. For cognitive function, a short-term memory test demonstrated that different developmental environments lead to differences in discrimination and preferences when given a choice between familiar and novel individuals. Thus, the neuronal numbers across the brain will provide a quantitative measure of how social environment influences brain development and adult social skills. This study will establish the effects of environmental complexity on brain development and behaviour in a complementary fashion by setting together different aspects of brain evolution.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: Ministerio de Ciencia, Innovación y Universidades, Spain (PSI2018-094627-R)
Premio UNED-Banco Santander-Investigación

Title: Gender differences in the association between loneliness and cortisol in older adults

Authors: ***C. VENERO**[1], S. GARCÍA-HERRANZ[1], S. BALIYAN[1], L. UTRERA[1], R. RODRÍGUEZ-FERNÁNDEZ[1], M. GEORGIADIS[1], R. VELASCO[1], P. SAMPREDRO-PIQUERO[2], A. VALENCIA[1], H. PERAITA[1], M. DÍAZ-MARDOMINGO[1];
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Abstract: Psychological and sociological factors have a significant impact on health and well-being. Thus, living an active lifestyle that involves participation in social activities has been related to better mental well-being. In contrast, loneliness, the subjective perception of social isolation even when one is accompanied by others, has been consistently associated with poor mental health, most prominently depression, and with poor physical health and mortality. Consequently, loneliness is considered an important contributor to physiological stress processes. In fact, the alteration in the circadian rhythm of the hypothalamic-pituitary-adrenal axis (HPA) has been proposed as a way in which loneliness influences health. The aim of the present study was to investigate whether higher levels and diurnal variation of salivary cortisol are cross-sectionally associated with loneliness in older adults without depression. Participants were administered the Social and Emotional Loneliness Scale for Adults (SELSA-S) to measure the feelings of social and emotional (family and romantic) loneliness. In addition, the Geriatric

Depression Scale was used to identify depression. Five samples of diurnal salivary cortisol (upon awakening, 30 minutes after waking up, 45 minutes after waking up, 8 hours after waking and before bedtime) were taken in a sample of 114 community-living older adults (>60 years old). Correlational analyses indicated that cortisol levels at awakening were positively associated to social loneliness. Stepwise multiple linear regression analyses were conducted to investigate whether cortisol levels predicted social and emotional loneliness. We observed that awakening cortisol levels predicted social, but not emotional loneliness. When gender was taken into account, this relationship was stronger in men, than in women. Interestingly, social loneliness was better predicted by cortisol levels in subjects living alone than in those living accompanied. Our results suggest that alterations in HPA axis activity are related to the degree of social loneliness.

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Poster

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Topic: F.02. Behavioral Neuroendocrinology

Support: Z01-MH-002498

Title: Stimulation of median raphe terminals in dorsal CA2 reduces social investigation

Authors: *S. LEE[1], S. WILLIAMS AVRAM[1], A. CYMERBLIT-SABBA[1], N. CILZ[1], J. SONG[1], K. COUREY[2], S. YOUNG[1];
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Abstract: The hippocampal cornu ammonis area 2 (CA2) region is essential for social memory. Recently, we showed that targeted CA2 stimulation of vasopressin presynaptic fibers from the paraventricular nuclei of hypothalamus strongly enhances social memory in mice (Smith *et al.*2016). In addition, the dorsal CA2 (dCA2) area of the mouse hippocampus receives neuronal inputs from other regions including the septal nuclei, the diagonal bands of Broca, supramammillary nuclei, and median raphe nucleus (Cui *et al.*2013). However, the functions of these neurocircuits have been scarcely investigated. Thus, it is important to pinpoint how these neuronal inputs to CA2 from different regions help orchestrate various behaviors that the CA2 may play a role, including social recognition, object recognition, aggression and anxiety-like behaviors. Serotonergic neurons play a role in anxiety-like and aggressive behaviors. Thus, we

investigated the behavioral role of presynaptic fibers from the median raphe nucleus projecting to the dCA2. We used a transgenic mouse serotonin transporter promoter (Slc6a4)-driven Cre recombinase line and injected AAV-EF1a-DIO-hChR2(H134R)-mCherry (ChR) virus into the median raphe nucleus followed by implantation of optic fibers into the dCA2. Slice recording of ChR injected median raphe showed light evoked action potential firing which confirmed ChR-induced excitability. The neuronal fibers containing ChR projecting from the median raphe nucleus to dCA2 were optogenetically stimulated in the dCA2 during various social behavioral tests. We observed no effects on social or object memory, or anxiety-like behavior, or investigation of physical objects; yet, there was a decrease in social interaction with novel ovariectomized female mice. Thus, serotonergic input may regulate sociability without affecting memory.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: 1R15HD075222-01A1
HHMIGRANT52008125
HRD-1810898

Title: Estrogen receptor alpha expression in the medial amygdala influences socially monogamous behavior of male prairie voles in a field setting

Authors: *S. A. CASTILLO[1], C. T. LAMBERT[2], B. KEANE[3], J. B. LICHTER[4], A. N. PERRY[6], B. S. CUSHING[7], N. G. SOLOMON[5];
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Abstract: Here we report the first findings directly linking site-specific estrogen receptor alpha (ERα) expression and expression of male behavior in the field associated with mating strategy. The mechanisms of monogamy have attracted special interest due, in part, to the rarity of monogamy in mammals and its occurrence in humans. Laboratory experiments have demonstrated the essential nature of ERαexpression, low levels, in regions such as the medial amygdala (MeA), in permitting the high levels of male prosocial behavior, pair bond formation

and parental behavior, associated with social monogamy. However, whether this translates within the complexity of the natural environment is unknown. Therefore, we tested the hypothesis that within ecologically relevant setting ERαexpression influences the monogamous behavior of male prairie voles (*Microtus ochrogaster*). Taking advantage of the fact that the F_1male offspring (KI) of Kansas female and Illinois male prairie vole mating display significantly lower levels of prosocial behavior than other male prairie voles and overexpress ERα, we created male in which we could reduce ERα expression. We established replicate breeding populations in semi-natural outdoor enclosures, 8 sexually naïve adult Illinois females and 8 adult sexually naïve KI males per enclosure. In half of the males the MeA had been transfected with an anti-ERα shRNA containing adeno-associated viral vector to inhibit ERα expression two weeks before release. Population were followed for 15 weeks and RNAScope®was used to demonstrate the effectiveness of the shRNA. As predicted ERα-shRNA males had stronger associations with a single female, smaller home ranges overlapping fewer females, and greater home range overlap with one female than control, high expressing ERα, males with higher ERα. Our findings suggest that ERα in the MeA is an ecologically relevant mediator of affiliative behavior and may be important in the evolution of monogamy in the prairie vole. We also usedRNAScope® to compare the expression of the vasopressin (V1a) receptors in the MeA and lateral septum of treated males. This was done because V1aR has been shown, in the lab, to also play a major role in expression of social monogamy in males and we wanted to determine if changes in ERαaffected V1a mRNA expression.

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Poster

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Topic: F.02. Behavioral Neuroendocrinology

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NIH Grant GM049011

Title: Neural and behavioral correlates of social attachments in juvenile prairie voles

Authors: A. R. ALAWNEH, *A. N. PERRY, B. S. CUSHING;
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Abstract: Social relationships are an important factor in determining overall mental and physical health. Relationships with family members and friends comprise a large proportion of the interpersonal interactions that satisfy our need for social connection, prevent feelings of

loneliness, and provide social stabilization during adolescence and adulthood; however, the majority of our knowledge on the neurobiology of social attachments has largely been derived from studies of maternal-infant attachments, pair bond formation between adult mates, and to a lesser extent adult peer attachments. Therefore, the objective of this research was to characterize sibling and peer attachments in juvenile male and female prairie voles (*Microtus ochrogaster*) to complement and extend what is known about non-sexual, peer attachments in adults. In our first experiment, we examined the stability of sibling bonds in the presence of a novel peer and predicted that juveniles would spend more time huddling with their familiar sibling, as has been demonstrated in adult voles. We then cohabitated juveniles with a same-sex peer for 24 hours to determine if they are capable of forming selective peer attachments based on familiarity. Preference tests were conducted to examine whether familiar peers were preferred relative to novel peers of the same or opposite sex. As juveniles are non-reproductive, we predicted that affiliation would be based entirely on familiarity regardless of the sex of the peer- unlike adults, where potential mates destabilizes same-sex peer attachments. In a second experiment, we examined the neural response to reunion with a sibling, familiar peer or novel peer following 24 hours of isolation. Using c-fos, a marker of neural activation, we predict that while social stimuli would activate neurons in the paraventricular nucleus of the hypothalamus (PVH) during the reunion phase that familiar individuals (sibling and peer) would preferentially activate oxytocin neurons, whereas the novel peer would activate other PVH cell types. We will present co-localizations findings. Our results indicate that juvenile attachments differ from adult attachments. Juveniles preferred to huddle with novel peers compared to their familiar siblings, but like adults do form selective attachments with a familiar individual. Juveniles appear to be predisposed to form attachments with novel individuals which may facilitate dispersal from the natal nest and ultimately find an unrelated mate. We will also report the findings from the neural activation study, which will help clarify how the brain processes social signals from familiar and unfamiliar conspecifics with different salience.

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Poster

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Topic: F.02. Behavioral Neuroendocrinology

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Title: Cloning and expression of the estrogen receptors α and β from the prairie vole (microtus ochrogaster)

Authors: *G. PENA CONTRERAS, K. R. HERNANDEZ, A. N. PERRY, M. MIRANDA-ARANGO, B. S. CUSHING;
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Abstract: There are two main Estrogen Receptors (ER), ERα and ERβ, which have distinct although overlapping distribution in the brain. They have been shown to play a critical role in the organization and expression of sociosexual behavior. The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent that forms long term pair bonds and displays biparental care, therefore making it an outstanding model system for the study of complex (and human-relevant) social behaviors. Previous studies have demonstrated that ERα is a critical determinant of prosocial behavior in male prairie voles. Specifically, reduced ERα expression in the medial amygdala and bed nucleus of the stria terminalis is necessary for the display of high levels of prosocial behavior. In contrast, the contribution of ERβ to prosocial behavior is less clear due in large part to the lack of available tools for studying this receptor (e.g., antibodies to visualize receptor expression via immunohistochemistry) and unlike ERα it does not appear to be sexually dimorphic in prairie voles. In the present study, we cloned the two vole ER genes and packaged them into adeno associated viral (AAV) vectors under the control of the CMV promoter and each with a distinct reporter gene (GFP and mCherry). These constructs have been expressed in HEK cells, and expression of the ER gene and its associated reporter was verified. Our goal is to demonstrate *in vivo* expression in the paraventricular nucleus of the hypothalamus (PVN) and expression effects, via co-localization on neurons in the PVN that produce vasopressin in prairie voles. It is anticipated that these AAV vectors allowing over-expression of vole-specific ERα and ERβ will be a great resource to further identify the contribution of each receptor subtype to the neuroendocrine regulation of prosocial behavior.

Disclosures: G. Pena Contreras: None. **K.R. Hernandez:** None. **A.N. Perry:** None. **M. Miranda-Arango:** None. **B.S. Cushing:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.21/Q4

Topic: F.02. Behavioral Neuroendocrinology

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NIH Grant 1R15HD075222-01A1
NIGMS Grant R25GM069621-09

Title: Awake BOLD response to predator odor reveals populational differences in emotionality and temperament in prairie voles

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Abstract: Prairie voles (*Microtus ochrogaster*) are the primary human-relevant rodent model for studying prosocial behavior, as they form long-term heterosexual bonds, display bi-parental care of offspring, and have cultural/populational differences in prosocial behavior. Prairie voles from Illinois (IL) are highly social while prairie voles from Kansas (KS) are significantly less social. Additionally, KS and IL males display significant differences in the expression of estrogen receptors, vasopressin, and oxytocin, which contribute to their distinct patterns of prosocial behavior. Cross-breeding studies have shown that in males, these differences are regulated by maternal influences, with male offspring of KS dams and IL sires (KI) displaying an exaggerated KS phenotype. While KS and IL prairie voles both form pair bonds, there are significant differences in the degree of their prosocial interactions, temperament, and emotionality. We have previously demonstrated that differences in gray matter microarchitecture between KI and IL males may contribute to behavioral differences in emotional expression and aggression. Based upon these differences, we hypothesized that awake blood oxygen level dependent (BOLD) responses in regions associated with the processing of predator odors and emotional coping strategies would differ between KI and IL males. We predicted that IL males would display robust activation in brain regions contributing to freezing and other passive coping mechanisms, while KI males would display a neural signature indicative of an overall more active coping strategy. Regions responsible for the processing of predator odor and fear responses include midline structures in the hypothalamus, thalamus, cortex, midbrain, and periaqueductal gray. We used awake functional magnetic resonance imaging to assess BOLD response to a predator odor in KI and IL males. Preliminary data suggests that when exposed to mountain lion urine odor stimulus, IL male voles responded with the predicted positive BOLD signal change in fear circuits, while KI males displayed a negative BOLD signal response in these regions and a positive BOLD response in regions associated with aggression. These results suggest that KI and IL males are emotionally distinguished and have distinctive temperaments. The results of this study provide a framework and model system for understanding cultural and populational differences in response to stressors and the expression of divergent coping strategies.

Disclosures: **R.J. Ortiz:** None. **J.R. Yee:** None. **P.P. Kulkarni:** None. **X. Cai:** None. **J.E. Mohl:** None. **A.N. Perry:** None. **C.F. Ferris:** Other; CFF has a financial interest in animal imaging research in the company that makes the vole imaging technology.. **B.S. Cushing:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.22/Q5

Topic: F.02. Behavioral Neuroendocrinology

Support: R15MH113085

Title: The role of reward signaling in prairie vole peer relationships

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Abstract: Relationships between same-sex peers are central to life in social groups. Prairie voles (*Microtus ochrogaster*) are widely studied for their reproductive pair-bonds, but individuals also demonstrate selective preferences for familiar same-sex peers. The mechanisms underlying reproductive pair-bonds in this species may differ from those underlying peer relationships, as reproductive partnerships and parental behaviors are highly motivated but peer relationships appear to be behaviorally less rewarding. Consistent with evidence of behavioral reward, blockade of dopamine or opioid receptors prevents the formation of prairie vole pair-bonds. However, blockade of dopamine receptors does not prevent the formation of peer relationships in the closely related but socially non-monogamous meadow vole (*Microtus pennsylvanicus*). We examined the role of reward and motivation in prairie vole peer relationships through pharmacological manipulations of dopamine and opioid signaling, and socially conditioned place preference. First, we determined that 6 hours of cohabitation with a new same-sex partner was insufficient for partner preference formation, and that 24 hours was sufficient for partner preference formation. Haloperidol bockade of dopamine receptors (with 24 hours of cohabitation) did not alter selective preferences for familiar same-sex partners, suggesting that dopamine neurotransmission is not necessary for the formation of prairie vole peer relationships, unlike mate relationships. However, activation of dopamine receptors with apomorphine (with 6 hours of cohabitation) induced partner preference. Prairie voles also exhibited socially conditioned place preference for same-sex peers, and this behavior was not blocked by haloperidol. These data support distinct roles of dopamine and motivation in peer relationships relative to pair-bonds: Although dopamine signaling is necessary to form pair-bonds, it is not necessary for the formation of a new peer relationship. Differences in the role of dopamine signaling in the formation of same-sex and opposite-sex social preferences suggest that reproductive bonds are mediated differently from non-reproductive ones, and thus that peer relationships need to be investigated separately from pair-bonds to fully elucidate mechanisms of social behavior.

Disclosures: **N.S. Lee:** None. **A.K. Beery:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Program #/Poster #: 069.23/Q6

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R15MH113085

Title: Social selectivity and social reward in prairie voles

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Abstract: In social species, relationships may form between mates, family members, and/or social peers. Prairie voles (*Microtus ochrogaster*) are socially monogamous rodents that form enduring and selective social preferences for a mate, as well as for familiar same-sex peers—allowing comparison of the properties of peer and mate relationships. Prairie vole mate bonds depend on dopamine signaling, unlike peer relationships, indicating potential differences in the role of behavioral reward in supporting these relationships. We conducted the first comparison of the reinforcement value of social interaction with familiar and novel same-sex and opposite-sex conspecifics, using operant conditioning with a social reward. Prairie voles were housed in pairs with a male or female partner (FF, FM, MM, MF). Voles were trained to lever press using a non-social (food) reward, then progressed to social testing. Each vole was tested in 30-minute daily sessions in a chamber in which lever pressing was reinforced by 1 minute of access to a second chamber with a tethered stimulus animal. Voles were tested for 8 days with their cage-mate partners and 8 days with novel, unfamiliar voles matched to the partner sex (order counterbalanced). Testing was followed by empty chamber and extinguishing controls. Both female groups (FF and FM) pressed at significantly higher rates for familiar partners than for unfamiliar strangers, with no difference between same-sex and opposite-sex breakpoints. In contrast, males in MF housing pressed more for social access than did males in MM pairs, with no effect of familiarity of the target on lever pressing effort. All subjects huddled extensively with partners but not with strangers, regardless of sex. Differences in subject motivation to access vs. huddle with unfamiliar conspecifics highlights important differences between social selectivity and social motivation. Sex differences in the social stimuli eliciting the greatest effort were striking, and are consistent with known, latent sex differences in mechanisms supporting social monogamy in males and females.

Disclosures: **A.B. Beery:** None. **J. Chen:** None. **N.S. Lee:** None. **S. Lopez:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant P50MH100023
NIH Grant R01MH115831
NIH Grant P51OD11132

Title: Pair bonding increases the predictability of the behavioral repertoire in prairie voles

Authors: *S. AGEZO[1], A. M. BORIE[1], K. JAIN[2], J. KWON[1], L. J. YOUNG[3], R. C. LIU[1], G. J. BERMAN[1];
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Abstract: Pair bonding is a complex social process that involves behavioral changes across contexts, including sociosexual interactions and parental care. A pair bond is thus a complex state that manifests itself across an animal's behavioral repertoire. Despite such complexity, this state is typically characterized with reference to only a few behaviors, often only one at a time. For example, in prairie voles, a canonical mammalian model of pair bonding, a bond is usually inferred only by observing one behavior, partner huddling, or another, resident-intruder attack. Here, we characterize how a more complete repertoire of natural behaviors is altered by the process of pair bonding. Specifically, we recorded the behavior of prairie voles both before and after a 24-hr cohabitation with another individual. Using a novel behavioral mapping technique, we measured the behavioral repertoire of male and female voles prior to and after pairing. In one group of voles, we paired a subject with an opposite-sex partner to establish a pair bond. In a second group, we paired a subject with a same-sex littermate as a non-bonded control. We quantified the behavioral repertoires expressed during a modified partner preference test. The modification prevented mating and huddling and helped ensure that the social approach could only be initiated by the subject. The resulting analysis allowed us to project all the animals' stereotyped behaviors into a common behavioral space. Qualitatively, we saw that regions within the map corresponded to distinct stereotyped behaviors. Quantitatively, inter-individual variability between behavioral maps (as measured by the Jensen Shannon divergence) increased after cohabitation in sibling-paired voles ($p < 10e-4$), suggesting that subjects typically become more different in how they act over time. In contrast, the behavioral repertoires of pair-bonded

voles became more similar to one another ($p < 10e-4$), suggesting more predictability in their behaviors - as if, paraphrasing Tolstoy, happy couples are all alike.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 069.25/Q8

Topic: F.02. Behavioral Neuroendocrinology

Support: Fondation pour la Recherche Medicale (FRM)
Agence Nationale pour la Recherche (ANR)
Fondation Jerome Lejeune

Title: Deciphering the combinatorial effects of septal oxytocin and vasopressin during social behavior in mice

Authors: *A. M. BORIE[1], F. MUSCATELLI-BOSSY[2], F. D. JEANNETEAU[3], M. G. DESARMÉNIEN[1];
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Abstract: Oxytocin (OT) and vasopressin (VP) modulate social behavior. Their roles have been interrogated in isolation whereas combinatorial effects are anticipated as both hormones are secreted during social interactions. The lateral septum (LS) is integrated in the social brain network where OT and VP neurons project axons and where both peptides are released during social interactions (Lukas et al. 2011, 2013). Reduced LS levels of OT receptor are associated with social deficits in *Magel2* knockout (KO) mice (Meziane et al 2015), a model of the Schaaf-Yang syndrome and to a lesser extent Prader Willi syndrome [genetic diseases with autistic features and social skills impairment]. **Aim**: Deciphering the combinatorial effects of septal OT and VP during social behavior. **Methods**: We used EEG theta activity to characterize OT/VP-dependent electrophysiological signatures and their sequence of actions during social interactions in male mice. We used pharmacology and optogenetic in WT and KO mice crossed in OT-CRE and VP-CRE background to manipulate OT and VP systems to LS during social recognition-discrimination paradigm. **Results**: In WT mice, septal VP and OT modify EEG theta activity, which signatures are specified during social novelty for VP and social habituation for OT. In KO mice, the septal VP EEG trace is absent during social novelty causing social discrimination defects restored by pharmacological and optogenetic stimulation of the septal VP system during social novelty. Pharmacological and optogenetic blockade of septal OT system during social

habituation impaired social discrimination in WT mice and prevented VP-elicited rescue of KO mice. **Conclusions**: The sequence of septal VP and septal OT determines social behavior in health and disease.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH grant P50 MH100023
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NIH grant P51 OD11132

Title: Sociosexual experience shapes oxytocin action on glutamaterigc transmission in the nucleus accumbens of prairie voles

Authors: *J. GUO[1,2], A. M. BORIE[1,3], S. AGEZO[1,3], P. LUNSFORD[1,3], L. J. YOUNG[1,2], R. C. LIU[1,3];
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Abstract: In socially monogamous prairie voles, sociosexual experience establishes enduring pair bonds between mates. Oxytocin (OXT) signaling in the nucleus accumbens (NAc) plays a crucial role in mating induced pair bonding. It is hypothesized that OXT signaling in the NAc links the neural encoding of partner cues to the reward system by modulating coordinated activity within a social salience network. Multiple brain regions, including prefrontal cortex and basolateral amygdala, send glutamatergic projections to NAc, and may contribute to the transition to a bonded state. However, how OXT and pair bonding affects communication within this network is unknown. Using *in vitro* electrophysiological recordings in prairie voles, we examined the effect of OXT signaling on excitatory transmission in the NAc of pair bonded and non-bonded voles. Animals were either cohabitated with an opposite-sex partner for 24 hours to establish a pair bond, or a same-sex sibling as a non-bonded control. In pair bonded animals, OXT receptor agonist, TGOT, did not alter the frequency or the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) in whole cell recordings. However, field excitatory postsynaptic potential (fEPSP) induced by local electrical stimulation of NAc was increased by TGOT relative to baseline. In contrast, in non-bonded voles, TGOT increased the frequency of

sEPSCs without changing their amplitudes, and modestly decreased fEPSP. Thus, OXT signaling affects excitatory transmission within the vole NAc differentially depending on sociosexual experience. Further, OXT signaling has different effects on spontaneous and evoked activity, perhaps modulating the signal-to-noise ratio in excitatory signaling in a state-dependent manner. Hence, our study suggests that sociosexual experience transitioning to a pair bonded state can reshape how OXT acts on glutamatergic transmission in the NAc, which may reflect a novel form of plasticity underlying pair bonding.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant P50MH100024
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Title: Ultrasonic vocalizations reflect discrimination of conspecific odors in pair-bonded male prairie voles

Authors: *D. ARSLAN[1,3], Y. J. KWON[2,3,4], L. J. YOUNG[3,4,5], R. C. LIU[1,2,3,4];
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Abstract: Ultrasonic vocalizations (USVs) are an important part of rodent communication, which have been studied extensively in mice and rats. Conspecific odors are a reliable way to elicit USVs, although research thus far has not revealed whether USV emission can reflect the recognition of conspecifics. Here we exploit prairie voles for their socially monogamous behavior, which presumably depends upon the ability to recognize one's partner. Like other rodents, prairie voles use olfactory cues for social communication, but whether these cues can elicit USVs differently for a partner versus a stranger is unknown. In our study, we first confirmed that olfactory cues can be used by male prairie voles to discriminate between their pair-bonded partner and a stranger. We performed a 2-alternative choice odor test in a 3 chambered cage, presenting a male subject with soiled bedding from its female partner or a stranger. Surprisingly, investigation time and time spent in each chamber was significantly longer for the stranger's bedding over the partner's, perhaps driven by arousal to a novel

individual's odor. Nevertheless, the result confirmed a differential recognition of the partner vs. the stranger cue. Next, in separate recording sessions, we presented males with urine or anogenital scent from the partner, a stranger female, a stranger male, or a water control. Similar to the investigation time in the 2-choice test, we found that USV emission to the female stranger scent was stronger than to partner scents. Furthermore, USV emission was more discriminative for anogenital scents compared to urine, and vocalizations elicited by social odors were all higher than for water control. Taken together, our results demonstrate that male prairie voles not only recognize the odors of their partner, but display that recognition in both their investigative and vocal responses to those odors. Whether emitted vocalizations might then be used by a receiver to recognize the partner is under current investigation. This work therefore lays a groundwork for studying the neural bases of learning to recognize the social-sensory cues of a partner.

Disclosures: D. Arslan: None. **Y.J. Kwon:** None. **L.J. Young:** None. **R.C. Liu:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIMH Grant 058616-R01
NIMH Grant 108527-R01
USDA-NIFA Grant 2014-67013-21579

Title: Probiotics and social behavior: Lacto*bacillus* reuteri administration affects social affiliation, neurochemical expression, and the gut microbiome in socially monogamous prairie voles

Authors: *M. L. DONOVAN[1], Y. LIU[1], G. N. PLATT[2], B. K. WASHBURN[2], M. LYNCH[3], T. C. CHARLES[4], J. T. CURTIS[5], K. M. JONES[2], Z. WANG[1];
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Abstract: Recent evidence for the regulatory role of gut microbiota in clinically relevant behaviors has grown exponentially. One species of bacteria in the gut, *Lactobacillus reuteri*, has been shown to alter a variety of behaviors, including social deficits - yet, the underlying mechanisms are still largely unknown. Although traditional lab rodents provide a strong foundation for the role of gut microbiota on behaviors, they do not naturally display a wide variety of social behaviors. The socially monogamous prairie vole (*Microtus ochrogaster*)

displays high levels of social behavior and thus provides an excellent opportunity to study influences of gut microbiota on social behaviors and the underlying mechanisms. Using prairie voles, we tested the hypothesis that administration of *L. reuteri* alters social and anxiety-like behaviors, neurochemical systems, and gut microbiota composition. Live or heated-killed (HK control) *L. reuteri* MM4-1A was administered into the drinking water of female voles daily for 4 weeks. Our data show that female voles treated with live *L. reuteri* MM4-1A displayed decreased social affiliation behavior and increased anxiety-like behavior compared to HK controls. Further, live *L. reuteri* treatment decreased corticotrophin releasing factor (CRF) and CRHR2 receptor expression in the nucleus accumbens (NAcc) and vasopressin 1a-receptor (V1aR) expression in the paraventricular nucleus of the hypothalamus (PVN), but increased CRF expression in the PVN in female voles. Our metagenomic sequencing data analyses indicate microbiome differences in both strain abundance and abundance of genes for utilization of complex polysaccharides relative to controls. Together, our data show behavior-, neurochemical-, and brain region-specific effects of live *L. reuteri* in female prairie voles. We are currently analyzing the effects of *L. reuteri* administration in male voles to examine potential sex differences.

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Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Support: NIMH Grant 058616-R01
NIMH Grant 108527-R01

Title: Stimulus-specific effects of social buffering and underlying neurochemical mechanisms in prairie voles

Authors: *E. K. CHUN, M. L. DONOVAN, Y. LIU, Z. WANG;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: Social support can help lower the stress response and its subsequent negative health outcomes - a phenomenon described as “social buffering”. These buffering effects can vary depending on both the type and intensity of the relationship, making the familiarity of the individual providing the support an important factor. In the present study we used the socially monogamous prairie vole (*Microtus ochrogaster*) model to examine the stimulus-specific

preventative social buffering effects on anxiety-like behavior induced by immobilization (IMO) stress. Female voles were divided into three groups that were exposed to an IMO restrainer tube containing a previously pair-bonded male partner (Partner), a male stranger (Stranger), or nothing (Control) for 60 mins, during which the females' behaviors were recorded. Following the 60-min test, anxiety-like behaviors were assessed for the IMO males using an elevated plus maze (EPM) test. Our data indicate that females in the Partner group spent significantly more time interacting with the restrainer than ones in the Stranger and Control groups. Control females showed increased levels of locomotor activity and rearing/self-grooming compared to the other two groups. Furthermore, IMO males that had a female partner in the arena entered the open arms more and tended to spend more time there in the subsequent EPM test, indicating decreased anxiety-like behaviors compared to IMO males that were exposed to a female stranger or just an empty arena. Interestingly, the time that females spent interacting with the restrainer was positively correlated with decreased anxiety-like behavior only for partners, but not for stranger males. These data not only reveal stimulus-specific social behaviors, but also indicate potential roles of those behaviors in buffering stress responses in prairie voles. We are currently analyzing neuronal activation via immediate early gene labeling to examine coordination between stress and social buffering neural networks. In addition, we are also assessing potential sex differences by exposing free-moving males to IMO-stressed females.

Disclosures: E.K. Chun: None. **M.L. Donovan:** None. **Y. Liu:** None. **Z. Wang:** None.

Poster

069. Neural and Contextual Modulation of Affiliative Behavior

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Topic: F.02. Behavioral Neuroendocrinology

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NIH grant P51OD11132
NARSAD YI grant 8211500004

Title: The development of AAV-CRISPR/Cas9 to edit the prairie vole genome in adult brain

Authors: *A. J. BOENDER[1], M. BOON[2], A. BORIE[1], L. J. YOUNG[1];
[1]Ctr. for Translational Social Neuroscience, Yerkes Nat. Prim. Res. Cntr, Emory Univ., Atlanta, GA; [2]Univ. of Groningen, Groningen, Netherlands

Abstract: Non-traditional model organisms provide excellent opportunities to investigate the neural mechanisms underlying diverse behaviors, but difficulty in the ability to manipulate their genome is a substantial impediment to research progress. Socially monogamous prairie voles

have proven useful to identify mechanisms regulating complex social behaviors. Pharmacological studies have revealed a role for oxytocin (OXT) signaling in modulating alloparental care, pair bonding and consoling behavior, yet little is known about how OXT influences specific cellular ensembles to regulate these behaviors. To better explore the circuit level mechanisms underlying social behaviors with precision, tools that enable selective targeting and disruption of the OXT receptor gene (*Oxtr*) in adult brain are needed. We recently used CRISPR/Cas9 genome editing to generate germline *Oxtr* mutant prairie voles. Here, we have developed an AAV-mediated CRISPR/Cas9 strategy to create genomic edits (indels) in *Oxtr* in adult vole brain. With a dual vector approach to deliver spCas9 and *Oxtr*-targeting sgRNA, we successfully mutated Oxtr genomic sequences in primary embryonic cultures. *In vitro editing* efficiency was assessed using the T7 endonuclease I assay and TIDE analysis, which showed editing efficiencies of ≈30%. Next, we injected both vectors or a control into the NAc of adult prairie voles and brains were taken 2 or 8 weeks after surgery. Transfected tissue was identified through eGFP fluorescence. Analysis of genomic *Oxtr* DNA suggested successful Cas9-mediated genome editing in transfected tissue. Finally, AAV-CRISPR/Cas9 infusion into the NAc led to a fairly robust (≈40%) decrease in I^{125}-OVTA binding after 8 weeks, demonstrating that this approach can be used to disrupt OXTR signaling in adult brain. Currently, we are adapting this strategy to allow for cell-type specific genomic editing using selective promoters, such that OXTR signaling can be specifically disrupted in astrocytic and/or neuronal populations.

Disclosures: **A.J. Boender:** None. **M. Boon:** None. **A. Borie:** None. **L.J. Young:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: F.04. Stress and the Brain

Support: JSPS KAKENHI Grant Number 15K09815
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Advanced Research on Military Medicine

Title: Effects of maternal separation stress on inflammatory cytokines in a rat model of post-traumatic stress disorder

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Abstract: Victims of child abuse are known to be at an increased risk of mental disorders such as depression and post-traumatic stress disorder (PTSD). The association with inflammatory cytokines has been examined as one of its biological conditions. In animal experiments, exposure to Early Life Stress (ELS) causes elevations of IL-1α, IL-1β, IL-6, TNFα, IL-12, IFNγ etc. Also, it has been pointed out that Adult Life Stress (ALS) could amplified changes of these cytokines.We have developed a rat model of PTSD using a shuttle box 2 weeks after inescapable stress (IS). In this model, maternal separation (MS) increased the number of learned helplessness behavior which was thought to be indicative of depressive symptoms. In this study, MS was taken as ELS, and IS was taken as ALS. We examined the effects of ELS and ALS on various cytokines in plasma extracted from MS (-) IS (-), MS (+) IS (-), MS (-) IS and MS (+) IS (+) groups. The cytokine of EPO, G-CSF, GM-CSF, GRO/KC, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL -6, IL-7, IL-10, IL-12, IL-13, IL-17, IL-18, M-CSF, MCP-1, MIP-1α, MIP-3α, RANTES, TNF-α, VEGF was comprehensively measured using Bio-Plex from BIO-RAD.When the above 4 groups were tested by 2-way ANOVA, the main effects of IS were significant in G-CSF, GRO/KC, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-7, IL -10, IL-17, MCP-1, MIP-3α, RANTES, and VEGF. The main effects of MS were significant in IL-7 and GRO/KC, and the interactions between MS and IS were significant in IL-7, IL-18 and GRO/KC. GRO/KC is also called chemokine (CXC motif) ligand 1 (CXCL1), and reported that the anti-inflammatory effect of imipramine involved the suppression of CXCL1 expression (Lee et al 2012) and CXCL1 promoted maturation to oligodendrocytes using neural precursor cells (Turbic et al, 2011). In our previous studies, MS suppresses the differentiation to neuron in neural precursor cells isolated from adult rat hippocampal dentate gyrus (Boku et al, 2015). The results of this study suggest that MS might suppress maturation to oligodendrocytes by reducing the expression of CXCL1.

Disclosures: H. Toda: None. **M. Tanichi:** None. **M. Koga:** None. **S. Tokuno:** None. **S. Takeshita:** None. **M. Nagamine:** None. **M. Fujita:** None. **K. Shimizu:** None. **A. Yoshino:** None. **T. Saito:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.02/Q15

Topic: F.04. Stress and the Brain

Support: KAKENHI 16K19189

Title: Preconditioning with lipopolysaccharide improves inflammation-induced depressive-like behaviors

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Abstract: Recent studies have indicated that preconditioning with lipopolysaccharide (LPS) reduces the subsequent damage that occurs following ischemic stroke and brain injury. Although a number of recent studies have suggested that neuroinflammation plays a role in depression, medication or preventive strategies focusing on inflammation have been poorly investigated in depression. We hypothesized that if inflammation significantly contributes to depression, that inflammation preconditioning would influence the symptoms. In the present study, we evaluated the effect of preconditioning with low-dose LPS on depressive-like behaviors induced by high-dose LPS-induced systemic inflammation in mice. Mice were administered two injections and randomly divided into four groups according to the pattern of the primary injection and secondary injection (6 days later) as follows: (1) saline & saline, (2) saline & high-dose LPS, (3) low-dose LPS & saline, and (4) low-dose LPS & high-dose LPS. Behavioral evaluation was performed using open-field and forced swim tests 24 hours after the secondary injection. Additionally, biochemical features were investigated by analyzing the expression of cytokine, glial, and oxidative stress-associated genes in the brain. Although low-dose LPS preconditioning did not affect spontaneous behavior in the open field, which was decreased by the high-dose LPS challenge, it markedly reduced immobility time in the forced swim test, which was increased by high-dose LPS. This suggested that preconditioning attenuates inflammation-induced depressive-like behaviors. The gene expression analysis showed that the responses to systemic inflammation differed in the presence or absence of preconditioning for several cytokines, glial, and oxidative stress-associated genes, suggesting that these genes are associated with the pathophysiology underlying the depressive-like behaviors. The present study showed that activation of the immune system contributes to improvement of depressive-like symptoms, and molecules involved in inflammation-induced depressive-like behaviors were identified.

Disclosures: **M. Koga:** None. **H. Toda:** None. **M. Kinoshita:** None. **F. Asai:** None. **Y. Mitsui:** None. **M. Nagamine:** None. **K. Shimizu:** None. **A. Yoshino:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.03/Q16

Topic: F.04. Stress and the Brain

Support: NSF IOS 1257679
NIMH MH106640
USD Governor's Research Center

Title: Adolescent social stress has protracted effects on neural and peripheral markers of inflammation

Authors: *M. J. WATT[1,2], M. SATHYANESAN[2], E. T. GRAACK[2], J. L. SCHOLL[2], M. A. WEBER[2], V. C. HUBER[2], G. L. FORSTER[1,2], S. S. NEWTON[2];
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Abstract: Teenage bullying is associated with greater incidence of psychiatric disorders and psychosomatic health problems in later life, which may be linked to compromised immune function. In support of this, former victims show elevated plasma C-reactive protein (CRP) decades after the bullying experience, while animal models of early life stress demonstrate increased expression of proinflammatory genes in cognitive and emotive circuits in the adult brain. However, whether chronic stress restricted to adolescence has long-lasting effects on central and systemic immune markers is not as well understood. We have shown that male rats exposed to repeated social defeat in adolescence exhibit learning/memory deficits as adults, along with disruptions to cortical and hippocampal monoamine activity. Here, we investigated whether adolescent defeat also results in alterations to neural and peripheral markers of immune function in adulthood. Plasma CRP levels were determined by ELISA, with hippocampal cytokine mRNA quantified using qPCR. Rats defeated in adolescence (postnatal day [P]35-39) displayed increased circulating CRP levels as adults (P60+), recapitulating clinical findings of protracted elevations in CRP of former victims of bullying. Changes in expression of proinflammatory cytokines were specific to discrete hippocampal subregions, with decreased interleukin-1β (IL-1β) and interleukin-6 (IL-6) evident in the dorsal dentate gyrus (DG), while reductions in interleukin-2 receptor β (IL-2Rβ), IL-6, and tumor necrosis factor α (TNFα) were seen in the ventral CA1. In contrast, rats defeated in adulthood (P63-67) showed increases in TNFα in the dorsal DG when sampled 3 weeks later, suggesting differential effects of social defeat on neuroinflammation according to age at which the stressor is experienced. We posit the decreases in hippocampal proinflammatory markers following adolescent defeat may contribute to previously observed deficits in adult contextual fear learning, given these cytokines are known to either promote hippocampal dependent memory or optimally modulate hippocampal plasticity under non-infected conditions. Combined, these findings suggest long lasting effects of adolescent social stress on basal immune markers that have been directly implicated in poorer psychological and physical health outcomes in later life.

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Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.04/Q17

Topic: F.04. Stress and the Brain

Support: This work was funded by a NARSAD Young Investigator Award by Brain and Behavior Research Foundation, honoured by P&S Fund (to NCG, Grant ID 25348)

Title: Stress primes secretory autophagy in the neuroimmune system

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Abstract: Immune and neuroendocrine systems play a major role in the regulation of brain homeostasis, a fundamental balance which impairment may lead to different neurodegenerative and neuropsychiatrtic diseases. A challenge to this homeostasis is environmental stress, which triggers an immediate stress response principally through the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the subsequent release of glucocorticoids (GC). The activation of the HPA can lead to enhanced inflammatory activity in the absence of infectious pathogens. The underlying mechanism, however, is unclear yet. In previous studies we showed that stress modulates macroautophagy via the co-chaperone and GR modulator, FKBP51. Given these findings, we investigated the possible role of stress on secretory autophagy, an unconventional secretory mechanism orchestrated by autophagic proteins, as molecular mechanism linking stress to neuroinflammation. Furthermore we tested our hypothesis for which the effect of stress on secretory autophagy is mediated via FKBP51. We adopted a translational approach starting by analyzing the molecular mechanisms *in vitro* in a microglia cell line, using dexamethasone, a synthetic glucocorticoid receptor antagonist, as stressor, and then analyzed *in vivo* the same effect after stressing the animals with foot shock. We discovered that FKBP51 is an indispensable player in the vesicle fusion for secretion. Through dexamethasone stimulation, we observed that FKBP51 mediates the effect of stress that causes an increased membrane fusion. With ELISA and microdialyses experiments, *in vitro* and *in vivo* respectively, we could show that the stress-enhanced activation of the membrane fusion complex leads to a significant increase in secretion of Interleukin-1B and Cathepsin D after stress in a FKBP51 dependent manner. To unravel novel proteins regulated by stress through secretory autophagy we preceded with a secretome analysis ad confirmed novel targets with biochemical assays. With this study

we show for the first time that stress enhances secretory autophagy via FKBP51, unravelling a possible mechanism that directly links stress to neuroinflammation.

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Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.05/Q18

Topic: F.04. Stress and the Brain

Support: JSPS KAKENHI Grant Number JP18K19262
JSPS KAKENHI Grant Number JP18KK0190

Title: Hypothermic responses under infectious condition depend on estrous stages in microsomal prostaglandin E synthase-1-deficient mice

Authors: *T. MATSUWAKI[1], M. ONISHI[1], A. FUJISAWA[1], K. YAMANOUCHI[1], A. BLOMQVIST[2], M. NISHIHARA[1];
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Abstract: Body temperature is maintained in a proper range by the central nervous system (CNS). This thermoregulatory homeostasis is disrupted by various kinds of stimulus such as immune challenges. Prostaglandin E_2 (PGE_2) is known as a strong pyrogen working on the hyperthermia-controlling region in the CNS under infectious condition. It has been demonstrated that mice lacking the inducible PGE_2 synthase, microsomal prostaglandin E synthase-1 (mPGES-1) show no endotoxin-dependent fever. In recent experiments from our group, however, some mPGES-1 deficient (KO) mice showed strong hypothermia in respond to injection of lipopolysaccharide (LPS). Thus, in the present study we performed detailed analyses of the thermoregulatory system in mPGES-1 deficient mice. First we examined the possible sexual difference of thermoregulatory responses to LPS in the wild-type (WT) and KO mice. We implanted a temperature transponder in the abdominal cavity 2 weeks before the experiment. LPS was intraperitoneally injected to the animals and body temperature was measured every minute. Both sexes of WT mice showed transient hyperthermia after LPS injection. On the other hand, no KO mice showed hyperthermia. Moreover, we found that female KO mice at proestrus stage showed a strong transient hypothermia, with the temperature dropping to 34°C or less about 5 hours after LPS injection. LPS did not affect body temperature of male KO mice or female KO mice at the other estrus stages. The concentration of sex steroids secreted from ovaries changes depending on the estrus cycle. Subsequently, we evaluated the effect of estrogen

on the LPS-inducible hypothermia in KO mice. We ovariectomized the KO mice 2 weeks before the experiment and treated a part of them with estrogen 24 hours before LPS injection. Ovariectomy blunted the hypothermic response of KO mice, which was restored by estrogen treatment. These results lead us to formulate the following hypotheses. 1) In the CNS, there is a hypothermia-controling area which downregulates the body temperature. 2) Hyperthermic and hypothermic centers in the CNS are simultaneously activated by immune signals. 3) Estrogen modulates the activity of the hypothermic center.

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Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.06/R1

Topic: F.04. Stress and the Brain

Title: Peripheral and central immune responses to trauma: Potential mechanisms in PTSD risk?

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Abstract: Background: Numerous studies support a role for inflammation as a potential pathophysiological mechanism underlying posttraumatic stress disorder (PTSD). In our prospective Marine Resiliency Study, high levels of plasma C-reactive protein (CRP) before deployment were associated with increased risk for developing PTSD after combat. However, the mechanisms underlying immune function and increased PTSD risk remain unclear. We hypothesized that behavioral susceptibility to trauma correlates with higher inflammatory response to trauma, and that the NLRP3 inflammasome modulates the behavioral and inflammatory effects of trauma. Here we examined the behavioral and inflammatory effects of trauma in the well-known predator stress model of PTSD.
Methods: Male and female mice were exposed, or not, to predator stress (exposure to a car for 10 min). Two weeks after the predator stress, the mice underwent a ”trauma-reminder test” in which their avoidance of cat litter was assessed. A composite avoidance score including the number of entries, as well as time spent and latency of first entry in the aversive area was calculated for each animal. Stressed mice were divided into “resilient” and “susceptible” groups based on the susceptibility cutoff score (0.5 SD at or above the mean of the non-stressed group). Mice were euthanized 24 hrs later, and plasma and brain tissues were collected. Plasma and brain levels of the pro-inflammatory and anti-inflammatory cytokines were assessed using ELISAs.
Results: Although stress increased brain and plasma CRP levels in males and females,

respectively, no difference was found between resilient and susceptible groups. In regards to the inflammatory response to stress, no significant difference was found in brain interleukin-1β (IL-1β) and IL-1 receptor antagonist (IL-1Ra) levels across all male and female groups. In males, susceptible animals exhibited elevated plasma IL-1β levels vs. resilient and control groups. Female susceptible mice showed decreased plasma IL-1Ra levels vs. resilient and control mice. Conclusions: These results show that the peripheral, not central, IL-1β/IL-1Ra pathway could contribute to trauma susceptibility. These findings suggest that targeting the immune signaling pathways might be a novel therapeutic alternative for PTSD patients. The effects of trauma in NLRP3-deficient mice will be presented, and the contribution of the NLRP3 inflammasome in brain-immune interactions, as well as blood-brain barrier integrity, in response to trauma will be discussed.

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Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.07/R2

Topic: F.04. Stress and the Brain

Support: Samsung Scholarship

Title: Effects of social experience on immune parameters and brain transcriptome in mouse social hierarchies

Authors: *W. LEE[1], J. P. CURLEY[2,1];
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Abstract: In social hierarchies, individuals occupy differential ranks based upon their history of competitive interactions. Following acquisition of ranks, adaptive changes in both behavior and physiology occur. Consequently, individuals of different social status experience vastly different social environments which may lead to health disparities. Chronic social subordination has been suggested to compromise individuals' immune systems due to the deleterious effects of elevated stress levels in both humans and non-human animals. Further, individual phenotypic differences

such as in copying style may lead individuals to being more or less resilient to this stress. In contrast, maintaining high social status, especially alpha status, can be extremely energetically costly as one invests vastly in reproduction and territorial defense and leaving less resources to fight immune challenges. For instance, our group has previously shown that alpha male mice living in social hierarchies invest vast resources in major urinary protein production and scent-marking and also consume food and water more frequently to match these metabolic costs. We have also shown that subordinate mice living in despotic social hierarchies, characterized by highly aggressive alpha males, exhibit higher level of basal corticosterone in blood. Based on our previous data, we hypothesize that both high- and low-ranked mice living in groups of 10 males show compromised immune systems in compared to mid-ranked mice associated with these metabolic trade-off and stress responses respectively. We collected biological samples (blood) from 10 groups of 10 outbred CD1 male mice housed in large vivaria constructed to resemble the wild habitat of the progenitors of laboratory mice. Samples were taken prior to group-housing and two weeks after group-housing. Plasma cytokine levels, plasma corticosterone levels, *fkbp5* gene methylation levels, and a characterization of the proportions of different types of immune cells in blood were assessed. Moreover, we collected in-depth behavioral data to examine whether individual variation in how animals respond to receiving aggression is associated with differences found in markers of immune system functioning. Lastly, we microdissected multiple brain regions involved in social decision making network and sequenced transcriptome. This study emphasizes the importance of considering variation in both individual behavioral phenotypes and social rank when studying the effects of differential social environments on the immune system within an ethologically relevant behavioral paradigm.

Disclosures: W. Lee: None. **J.P. Curley:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.08/R3

Topic: F.04. Stress and the Brain

Support: Brain Health Institute, Rutgers University Grant R21MH108994
NIH Grant 1K12GM093854

Title: Chronic stress affects microglial chemotactic activity in WT and NOP KO mice *in vivo*

Authors: *V. L. DIBONA[1], B. PENG[2], M. NISSENBAUM[1], H. ZHANG[1], U. EYO[3], L.-J. WU[4], M. ANSONOFF[2], J. E. PINTAR[2], A. W. KUSNECOV[5];
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Abstract: Microglia are known as the resident "immune" cells of the brain and have great similarity to macrophages. Functionally, microglia produce immune-related cytokines and engage in chemotaxis to injury sites and phagocytosis of cellular material. Under normal physiological conditions, microglia are highly dynamic with many ramified/branched processes that continually survey the CNS and assess neuronal health and homeostasis. Following injury or illness, microglia are quickly transformed from their surveillance state into an amoeboid, immune activated morphology with enlargement of the cell body and retraction and thickening of processes. Chronically activated microglia have been identified under conditions of physical damage, neuronal degeneration and/ or neurodevelopmental disorders. More, a number of studies have shown that psychological stressors, such as restraint, can impose changes in microglial morphology, and in turn, are associated with changes in working memory. NOP, the receptor for the neuropeptide OFQ/N, has been implicated in the regulation of anxiety-like responses, yet it's role in microglial responses to chronic stress is unknown. In the current study, we sought to determine whether a two-week chronic unpredictable stress (CUS) regimen alters real-time chemotaxis responses of microglia following a focal laser injury in the absence of NOP-1. To this end, we produced litters of C57Bl6/J $NOP^{+/+}$ $CX3CR1^{GFP/+}$ and $NOP^{-/-}$ $CX3CR1^{GFP/+}$ mice from appropriate matings. Young adult male mice were subjected to a 14-day mild CUS during which mice either remained in the home cage (Control condition) or were subjected to 4hrs/day variable stressor exposure. The overall design was as follows: WT/Control (N=6), NOP/Control (N=3), WT/Stress (N=3), NOP/Stress (N=4). Twenty-four hours after the final stressor, mice were anesthetized and a craniotomy performed to enable visualization of GFP^{+} cortical microglia using a 2-photon microscope. Repeated measures ANOVA revealed a significant interaction between time and stressor exposure ($p < 0.001$), with a borderline influence imposed by genotype ($p=0.088$). Stressed mice showed a substantially reduced microglia response (> 50% less fluorescence intensity) at the injury site, with the lowest responses observed for stressed $NOP^{-/-}$ mice, when compared with $NOP^{+/+}$ mice. While preliminary, these data support the possibility that chronic stressor exposure affects the dynamic response of microglia to cellular injury, and further suggests that the NOP system may be required for optimal microglial responses.

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Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.09/R4

Topic: F.04. Stress and the Brain

Support: USD Center for Brain and Behavior Pilot Grant

SD Governor's Research Center
Great Plains IDeA CTR NIH U54 GM115458
SPURA NIH R25 DA033674

Title: Methylation of genes and regulation of inflammatory processes in college students with alcoholic parents

Authors: *J. L. SCHOLL[1], K. PEARSON[2], K. A. BROWN-RICE[3], N. A. KALLSEN[4], G. E. DAVIES[4], E. A. EHLI[4], S. OLSON[2], A. SCHWEINLE[2], K. A. FERCHO[1], L. A. BAUGH[1], G. L. FORSTER[5];
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Abstract: The experience of trauma can have a significant and long term effect on a variety of mental health measures including depression, anxiety, post-traumatic stress disorders (PTSD), and enhanced risk of drug and alcohol abuse. Psychological health is also closely linked to neural function, stress hormones and inflammation responses. A significant proportion of college students are adult children of an alcoholic parent (ACoA), which can confer increased risk of traumatic experiences as well as heritable and environmental epigenetic modifications to the genome. The goal of this study was to better understand the relationships between psychological function, inflammation and epigenetic modifications and structural differences in cortical areas and how these measures influence resilience/vulnerability to substance use in ACoAs. To do so, measures of psychological health were assessed in resilient (not engaged in hazardous alcohol use) and vulnerable (currently engaged in hazardous alcohol use) ACoA college students. Activity within regions of interest (ROIs) (as measured by functional magnetic resonance imaging) and biological markers of chronic inflammation, cortisol and gene methylation in candidate genes associated with inflammatory response, glucocorticoid regulation and emotion and stress reactivity were compared between resilient and vulnerable groups. College ACoAs currently engaged in hazardous alcohol use reported more anxiety, depression and posttraumatic stress symptoms, and increased plasma C - reactive protein (CRP) as compared to ACoAs resistant to problem alcohol use. Vulnerable ACoAs also exhibited greater activity in the right hippocampus and ventral anterior cingulate cortex in response to emotional cues as compared to resilient ACoAs. Preliminary findings suggest differing methylation on promoter and CpG island regions of CRP and glucocorticoid-related genes that are thought to influence regulatory control on serum CRP, glucocorticoid receptor function and influence emotional processing, PTSD and anxiety. Taken together, these findings suggest a complex association between epigenetics, inflammatory regulation and psychological function that may be used in the future for early identification and intervention for mental health in ACoAs.

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Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.10/R5

Topic: F.04. Stress and the Brain

Support: NIH grant MH117482

Title: Central immune alterations in a gestational stress model of postpartum depression

Authors: *B. LEUNER[1], C. GOODPASTER[2], N. DEEMS[1], R. GILFARB[1], K. LENZ[1]; [1]Ohio State Univ., Columbus, OH; [2]The Ohio State Univ., Columbus, OH

Abstract: Postpartum depression (PPD) affects at least 15% of all new mothers. Although peripheral immune changes have been linked in some studies to PPD, little is known about how the brain's immune system is modified in PPD. To address this gap, we used a gestational stress rodent model of PPD that recapitulates the critical behavioral symptoms found in depressed human mothers along with neural remodeling (i.e. dendritic spine loss) in brain areas relevant to PPD including the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). Pregnant Sprague-Dawley rats were subjected to either chronic variable stress from gestational days (GD)7-20 or remained unstressed. Animals were sacrificed one day before (GD21) or one week after (postpartum day 8, PD8) parturition and brain tissue collected for qPCR to assess mRNA expression of several pro-inflammatory cytokines [interleukin (IL)-1B, IL-6, and tumor necrosis factor alpha (TNFα)] and markers of microglial phagocytosis [CD68, integrin alpha M (ITGAM), complement component 3 (C3), and complement component 1 (C1q)]. A separate cohort of stressed and unstressed mothers was perfused on PD8 and immunohistochemistry for Iba-1 performed to quantify microglial density and number. Our results show increases in microglial density and number in the mPFC and NAc of stressed mothers on PD8. Gestational stress also produced region- and timepoint-specific effects on the expression of pro-inflammatory cytokines and microglial phagocytic markers. Overall, our results indicate that gestational stress impacts the peripartum neuroimmune environment which could have important implications for understanding the mechanisms underlying neural and behavioral abnormalities in PPD.

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Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.11/R6

Topic: F.04. Stress and the Brain

Support: National Sciences and Engineering Council Grant 211075-190799-2001

Title: Effects of environmental enrichment and social housing on LPS-induced pubertal immune response

Authors: *K. B. SMITH[1], M. MURACK[1], R. CHANDRASEGARAM[2], D. TATA[3], J.-F. MALLET[1], C. MATAR[1], N. ISMAIL[1];
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Abstract: Puberty is a developmental period consisting of neuronal remodeling, and reorganization. occurring through accelerated neurogenesis and synaptic pruning. Rapid neuronal restructuring is sensitive to experience-dependant changes, such as stress. Pubertal stress-exposure results in sexual, cognitive, and mood disorders. Enduring stress-related maladies disproportionately affect females, rather than males. Currently, it is unclear how great an effect the environment has on the immune system following stress exposure. We investigated the effects of environment following an immune challenge in pubertal male and female mice. Mice arrived at 3 weeks of age and were immediately separated into either deprived (DH), social (SH) or environmentally enriched (EE) housing groups (N=60). At 6 weeks of age (puberty) mice were injected with lipopolysaccharide (LPS), a bacterial endotoxin and monitored for sickness behaviour. RT-qPCR analyzed mRNA levels of cytokines IL-1β, IL-6 and TNFα. Concentrations of cytokines, IFγ IL-1β, IL-6, IL-10, and TNFα. EE-housed male and female mice demonstrated a significant increase in sickness behaviour at 2, 4 and 6 hours following LPS treatment compared to the DH condition. Significantly greater sickness symptoms were seen in EE-housed females at the 2- and 4-hour mark compared to EE-housed males. EE-housed female mice expressed greater mRNA levels of hypothalamic IL-6 and TNFα compared to DH female mice. Additionally, IL-1β and TNFα mRNA expression was greater in DH females compared to DH males. These findings suggest that DH mice may be exhibiting a hypo-stress response to LPS as a result of their housing condition. Additionally, identification of sex differences supports previous findings indicating the distinct experiences to stress between males and females. Furthermore, this study provides new evidence exemplifying the substantial environmental contribution to well-being. The dramatic impact of a single stressor underscores the vulnerability of the pubertal period to stress.

Disclosures: **K.B. Smith:** None. **M. Murack:** None. **R. Chandrasegaram:** None. **D. Tata:** None. **J. Mallet:** None. **C. Matar:** None. **N. Ismail:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.12/R7

Topic: F.04. Stress and the Brain

Support: Lallemand Inc.
University of Richmond

Title: An investigation of a probiotic-supplemented diet on stress responsivity and immune function in male Long-Evans rats

Authors: *N. NATALE, B. HINDI, P. SANTORE, M. H. KENT, K. G. LAMBERT; Psychology and Neurosci. Dept., Univ. of Richmond, Richmond, VA

Abstract: Recent evidence suggests that gut microbes modulate the activity of the central nervous and endocrine systems by producing anxiolytic and antidepressant agents (Bravo et al., 2011; Dinan et al., 2013). To further examine the microbiome-gut-brain axis, the current study investigated the effects of a probiotic formula on neurobiological responses to acute stress in male rats. Twenty-four male Long-Evans rats were randomly assigned to a probiotic group (PB), milk fat control group (MF), or maltodextrin control group (n=8 per group). It was hypothesized that rats consuming *Lactobacillus helveticus* and *Bifidobacterium longum* would exhibit reduced markers of heightened anxiety, as well as faster wound healing. Behavioral analyses indicated that the PB group exhibited increased vigilance in the open field in the presence of predator scent exposure. Additionally, during the uncertainty challenge task, the PB group spent more time in proximity to the curtain, which is recognized as hyper-vigilant behavior. Following the behavioral tests, a small dermal punch was made to the rat's intrascapular area so that wound healing could be assessed; results indicated that the PB group's wounds healed faster than the other groups on the ninth day following the dermal punch. Brain immunohistochemistry was used to evaluate brain-derived neurotrophic factor (BDNF), microglia, and 5-HIAA serotonin metabolite immunoreactivity, while dehydroepiandrosterone (DHEA) and corticosterone (CORT) metabolites were assayed from fecal samples. The PB group had significantly higher BDNF levels in the hippocampus CA3 area, suggesting heightened neuroplasticity compared to the control groups. Further, the PB group had significantly lower 5-HIAA levels in the CA3, which is consistent with previous research (Desbonnet et al., 2010). In sum, these behavioral and neurochemical results support the hypothesis that probiotic treatments promote markers of emotional resilience in rats.

Disclosures: **N. Natale:** None. **B. Hindi:** None. **P. Santore:** None. **M.H. Kent:** None. **K.G. Lambert:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.13/R8

Topic: F.04. Stress and the Brain

Title: BDNF controls NRF2-KEAP1 through a receptor independent mechanism

Authors: F. BROUILLARD[1], J. FATH[1], A. CABAYÉ[2], D. CLAVERIE[3], G. TRUGNAN[4], C. BERNARD[5], *J.-J. BENOLIEL[6], **C. BECKER**[1];
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Abstract: The NRF2-KEAP1 pathway is a key regulator of oxidative stress, metabolism and inflammation. Understanding how KEAP1 represses NFR2 is essential to design therapies against numerous pathologies, including cancers and neurological disorders. Low Brain Derived Neurotrophic factor (BDNF) levels after an intense stress prevents the activation of the NRF2 pathway resulting in vulnerability to depression. BDNF appears as a novel key regulator of the KEAP1-NRF2 pathway in the brain, since *in vivo* injection of BDNF or BDNF siRNA increases or decreases NRF2 nuclear translocation, respectively. Our goal was to unravel the mechanisms underlying the action of BDNF on NRF2. We used hippocampal HT-22 cells to probe the mechanism. We demonstrate that brain derived neurotrophic factor (BDNF) constitutively competes with NRF2 on KEAP1 in neurons to ensure a basal level of nuclear translocation of NRF2. The presence of a cell-penetrating peptide sequence allows BDNF to freely cross the membrane to act on the NRF2-KEAP1 complex independently from its cognate TrkB and p75 receptors. We propose that the BDNF-NRF2-KEAP1 pathway constitutes a key regulator of cell homeostasis. Targeting the here identified sites of interaction between BDNF and KEAP1 provides new therapeutic possibilities for diseases characterized by inflammation, oxidative stress and low BDNF levels.

Disclosures: **F. Brouillard:** None. **J. Fath:** None. **A. Cabayé:** None. **D. Claverie:** None. **G. Trugnan:** None. **C. Bernard:** None. **J. Benoliel:** None. **C. Becker:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.14/R9

Topic: F.04. Stress and the Brain

Support: This research was supported by grant (No. 173014) from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Title: Chronical treatment with sertindole, but not clozapine and ziprasidone, afects the activity of antioxidative enzymes in rat brain

Authors: *N. TATALOVIC[1], A. NIKOLIć-KOKIć[1], Z. OREŠčANIN-DUŠIć[1], M. SPASIć[1], D. BLAGOJEVIć[1], &. MILJEVIć[2];
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Abstract: Introduction: Atypical antipsychotic drugs (APDs) are being used to treat acute psychotic episodes in schizophrenia and schizophrenia-related diseases as well as a variety of nonpsychotic disorders. Despite their effectiveness, these drugs have been associated with adverse effects on organs other than brain. We showed that APDs, such as clozapine (CLO), ziprasidone (ZIP) and sertindole (SER), increase oxidative stress and reduce antioxidative defence capacity in rat kidneys and heart. Given the scarcity of data about CLO, ZIP and SER actions on the brain redox homeostasis, the goal of current study was to investigate their impact on antioxidant enzymes.**Materials and Methods:** This study assessed activities of antioxidant enzymes: total superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in brain sonicates of 3 month old male Wistar rats treated daily via intubation with water (control group), CLO (45 mg/kg/day), ZIP (20 mg/kg/day) or SER (2.5 mg/kg/day) for 4 weeks. The procedures complied with directive 2010/63/EU regarding protection of animals used for experimental purposes.**Results:** There were no significant changes in investigated parameters in CLO and ZIP groups. However, in SER group the total SOD activity was decreased while CAT and GPx activities were increased compared to control group (One-way ANOVA with Tukey's HSD test, $p<0.05$).**Conclusion:** Since both CAT and GPx catalyze decomposition of H_2O_2, increased activity of these enzymes in SER group suggests an elevated concentration of their substrate in brain. Furthermore, the activity of SOD can be decreased by elevated concentration of H_2O_2 which additionally indicates the presence of increased quantities of this reactive oxygen species. These suggest that SER could cause a redox imbalance in brain with possible negative effects on mitochondrial respiration and neural tissue metabolism.

Disclosures: **N. Tatalovic:** None. **A. Nikolić-Kokić:** None. **Z. Oreščanin-Dušić:** None. **M. Spasić:** None. **D. Blagojević:** None. **&. Miljević:** None.

Poster

070. Stress and the Inflammatory/Immune Response

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 070.15/R10

Topic: F.04. Stress and the Brain

Support: CIHR Project grant to N.M.

Title: Neuroinflammation and blood-brain barrier integrity in depressed suicides with or without a history of child abuse

Authors: *M. WAKID[1], D. ALMEIDA[1], Y. WANG[2], M. DAVOLI[1], I. RAGOUSSIS[2], G. TURECKI[1], N. MECHAWAR[1];
[1]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]Genome Quebec, Montreal, QC, Canada

Abstract: Introduction: Child abuse (CA) is one of the most robust and reproducible predictors of major depressive disorder (MDD) in humans[1] and susceptibility to stress-related pathology may be due to key differences in the inflammatory response to stress[2]. The aim of this project is to examine whether a history of child abuse is associated with changes in the expression of selected neuroinflammatory and blood-brain barrier (BBB) markers. **Methods**: Expression of gene candidates using qPCR was quantified in well-characterized postmortem ventromedial prefrontal cortex (vmPFC) samples obtained from the Douglas-Bell Canada Brain Bank. These samples came from healthy 35-55 year-old male and female depressed suicides (with or without a history of severe CA) and matched sudden-death controls with no history of psychiatric nor neurological conditions (n=26/group). **Results**: A preliminary screening of a subset of relevant genes indicated a significant downregulation of fibroblast growth factor 2 (FGF2) in depressed suicides (20.7% and 33.6% decrease in abuse vs controls and in non-abuse vs controls, respectively, $p = 0.001$). **Discussion**: FGF2, a growth factor primarily expressed by astrocytes, stimulates growth, migration, and sprouting of endothelial cells and has been implicated in BBB integrity[3]. We speculate that the downregulation of this gene may reflect a compromised state of the BBB, as previously reported[4] and that it could be related to our previous report of increased macrophages surrounding blood vessels in the dorsal anterior cingulate cortex of depressed suicides[5]. A more high-throughput approach using the BIOMARK HD system is currently underway to quantify in the same samples the expression of 96 gene candidates representing key aspects of neuroinflammation and BBB integrity. These results will be presented together with FISH validation (RNAscope®) of some of the most differentially expressed genes.
Funding: CIHR Project grant to N.M.

1) Negele et al., 2015. *Depress Res Treat* **2)** Baumeister et al., 2016. *Mol Psychiatry* **3)** Bendfeldt et al., 2007. *J Neurosci* **4)** Menard et al., 2017. *Nat Neurosci* **5)** Torres-Platas et al., 2014. *Brain Behav Immun*

Disclosures: **M. Wakid:** None. **D. Almeida:** None. **Y. Wang:** None. **M. Davoli:** None. **I. Ragoussis:** None. **G. Turecki:** None. **N. Mechawar:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.01/R11

Topic: F.07. Autonomic Regulation

Support: FAPESP - grant#2018/00191-4
NEPAS

Title: Intravesical pressure changes evoked by angiotensin-(1-7) into the lateral preoptic area are similar to peripheral administration responses in female Wistar rats

Authors: G. B. LAMY[1], B. DO VALE[1], R. L. DE ALMEIDA[1], B. B. ANTONIO[1], D. P. VENANCIO[1], J. S. DE SOUZA[2], G. GIANNOCCO[2], ***M. A. SATO**[1];
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Abstract: Background: Chemical lesions of the lateral preoptic area (LPA) enhance water intake induced by subcutaneous injection of hypertonic saline in rats. In contrast, the LPA section abolishes the increase in intravesical pressure (IP) induced by olfactory tubercle stimulation in dogs. Immunohistochemical labeling has demonstrated the presence of Mas receptors for angiotensin-(1-7) in LPA. It is unknown whether neurons with Mas receptors for angiotensin-(1-7) in LPA are involved in urinary bladder regulation. Aim: To investigate the effects of angiotensin-(1-7) into the LPA and also the changes evoked by in situ administration on urinary bladder of angiotensin-(1-7) or intravenously (i.v.) on intravesical pressure in anesthetized female rats. Materials and methods: Female Wistar rats (~240 g, N=6, approved by Animal Ethics Committee- CEUA- protocol # 07/2015) underwent a stereotaxic surgery for implantation of stainless steel guide cannulas into the LPA under ketamine and xylazin anesthesia. After 7 days, the animals were anesthetized and maintained with 2% isoflurane in 100% O_2 and submitted to cannulation of the femoral artery and vein, placement of a miniaturized Doppler flow probe around the left renal artery to measure the renal blood flow and cannulation of the urinary bladder for IP measurements. After baseline IP and cardiovascular parameters recordings for 15 min, angiotensin-(1-7) (5 nmol/ uL) or saline (1 uL) was injected into the LPA and the

variables were measured for additional 60 min. Angiotensin-(1-7) 10 ng/0.1 mL (in situ on urinary bladder) or 0.24 ug/kg/min (i.v.) or saline (0.1 mL) administration were carried out and IP was measured for additional 90 min. Data are as mean±SEM (Paired Student t-test, $P<0.05$). Results: Unilateral injection of angiotensin-(1-7) into LPA evoked a significant increase in IP (187.5±37.2%) compared to saline (-2.1±1.9%). In situ angiotensin-(1-7) increased IP (147.4±18.9% vs. 3.2±2.8% saline), and i.v. angiotensin-(1-7) similarly increased IP (115.8±28.6%) compared to saline (-2.9±1.3%). No cardiovascular changes were observed after angiotensin-(1-7) or saline into the LPA or peripherally. Conclusion: The data suggest that LPA is an important part of the circuit that regulates the urinary bladder activity mediated by angiotensin-(1-7) and this peptide also evokes similar increases in IP after peripheral administration.

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Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.02/R12

Topic: F.07. Autonomic Regulation

Support: Grants-in-Aid for Scientific Research KAKENHI 25893280
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the Ministry of Education, Culture, Sports, Science and Technology S1411041

Title: TRPA1-expressing lamina propria mesenchymal cells regulate colonic motility through prostaglandin release

Authors: Y. YANG[1,2], S. WANG[1,3], K. KOBAYASHI[2], H. KANDA[1], Y. KOGURE[1], S. YAMAMOTO[1], H. YAMANAKA[2], K. NOGUCHI[2], *Y. DAI[1,2];
[1]Hyogo Univ. of Hlth. Sci., Kobe, Japan; [2]Hyogo Col. of Med., Nishinomiya, Japan; [3]Beijing Univ. of Chinese Med., Beijing, China

Abstract: Colonic motility including a peristalsis and mass peristalsis is one of the most important physiological activities for the digestion and absorption of nutrients. Regarding the mechanism of intestinal motility for small intestine, recent report showed the enterochromaffin (EC) cells use TRPA1 for chemo-sensing and stimulate intrinsic neurons to regulate intestinal motility and local contractile reflexes. However, localization of EC cell in colorectum is largely distinct from that in the small intestine. Molecular mechanisms underlying the colonic motility remain incompletely understood. In this study, we identified important role of TRPA1-

expressing mesenchymal cells in lamina propria for colonic motility. Furthermore, we investigated the mechanisms of abnormal colonic motility in pathological condition using an inflammatory bowel disease model. We found colorectal mesenchymal cells rather than EC cells express TRPA1 using *in situ* hybridization. Intra-colonic application of AITC, a TRPA1 agonist, produced intense colorectal contraction. To determine which substance to use for interaction between mesenchymal cells and intrinsic neurons, we used a human fibroblast cell line. This cell line constitutively express TRPA1 and we observed that these cells released PGE2 after TRPA1 activation. Moreover, application of PGE2 induced the colonic contraction and EP1/2 receptor antagonist blocked the AITC-induced intense contraction. These results suggested that colorectal mesenchymal cells may have interaction with intrinsic neurons and regulate the colonic motility. To further investigate the role of TRPA1 in abnormal colonic contraction in pathological condition, we used dextran sodium sulfate (DSS)-induced colitis model. DSS model showed abnormal colonic motility with intense contractions in wild type mice but not *Trpa1*-KO mice, compared with rhythmic colonic contractions in control animals. We also found that TRPA1 as well as its endogenous agonist 4-HNE were highly up-regulated in the colonic lamina propria in the DSS animals. In conclusion, these data suggest that TRPA1-expressing mesenchymal cells in mucosa may interact with intrinsic neurons through PGE2 release to regulate the colonic motility.

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Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.03/R13

Topic: F.07. Autonomic Regulation

Support: Medtronic Grant

Title: Real time closed loop control of bladder function with dorsal root ganglia sensory feedback and sacral root electrical stimulation

Authors: *Z. OUYANG, Z. J. SPERRY, E. C. BOTTORFF, T. M. BRUNS;
Biomed. Engin., Univ. of Michigan, Ann Arbor, MI

Abstract: Overactive bladder (OAB) affects millions of people worldwide. Patients suffer from a frequent and uncontrollable urge to urinate, which can lead to a poor quality of life. Current sacral neuromodulation therapy uses open-loop electrical stimulation to alleviate symptoms. In this study, we aim to improve therapy by developing a conditional stimulation paradigm using

neural recordings from dorsal root ganglia as sensory feedback. Stimulation is only given when an increase in bladder pressure is detected. Patients can potentially benefit from improved stimulation efficacy, a reduced occurrence of neural habituation over time, and a longer battery life. Experiments were performed in acute, anesthetized felines, in which the first and second sacral-level dorsal root ganglia (DRG) and roots were exposed bilaterally. A bipolar cuff electrode was placed on the left or right S1 root distal to the DRG for stimulation. One or two Utah arrays were implanted in the opposite S1 and/or S2 DRG. We implemented a Kalman filter-based model to estimate the bladder pressure in real-time using threshold crossings from the DRG recordings. The Medtronic Summit research development kit was used to control stimulation applied by an RC+S neurostimulator connected to the cuff electrode. Closed-loop neuromodulation was performed during continuous cystometry or when the bladder was partially full and non-voiding contractions were present. S1 stimulation at 5 Hz (200 μs pulse width, 1-2 times motor threshold) decreased the peak amplitude of non-voiding contractions by 63% and increased bladder capacity by 18-23% when applied continuously, in preliminary experiments. We observed that a model-estimated 6 cmH2O pressure increase within 4 seconds was effective at identifying 100% of non-voiding contractions, and used that threshold to initiate conditional stimulation. Non-randomized closed-loop stimulation trials increased bladder capacity by 26% compared to no stimulation in one experiment. This study demonstrates the utility of decoding bladder pressure from neural activity for closed-loop control. In the future, real-time validation during behavioral studies is necessary prior to clinical translation. Financial Support: Medtronic

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Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.04/R14

Topic: F.07. Autonomic Regulation

Support: Galvani Bioelectronics, UK.

Title: Stimulation of the pudendal sensory nerve alters voiding behavior in conscious unrestrained Wistar rats

Authors: *C. L. LANGDALE[1], J. A. HOKANSON[1], D. DEGOSKI[1], P. MILLIKEN[5], W. M. GRILL[1,2,3,4];
[1]Biomed. Engin., [2]Electrical and Computer Engin., [3]Neurobio., [4]Neurosurg., Duke Univ., Durham, NC; [5]Galvani Bioelectronics, Stevenage, United Kingdom

Abstract: Overactive bladder (OAB), resulting in urgency, frequency, and incontinence, is a highly prevalent condition that leads to medical complications and decreased quality of life. While studies investigating the effect of pudendal (Pud) nerve stimulation (Stim) have shown promising results clinically, effective Stim parameters to reduce OAB symptoms remain unclear. Recently, we observed that the effect of pudendal sensory (PudS) Stim on bladder capacity in acute-anesthetized rats was dependent on both frequency and amplitude. Our goal was to quantify the effect of PudS Stim on voiding behavior in conscious unrestrained Wistar rats. Food and water consumption, body weight, voiding frequency (VF), and voided volume (VV) were recorded. After habituation in metabolism cages, A 300 µM bipolar Stim cuff (connected to a skull-mounted headcap) was implanted on the left PudS branch. After recovery, animals were placed into metabolism cages three days a week (Pre-stim: Mon., Stim: Wed., and Post-stim: Thur.). On the day of Stim, impedances were recorded and Stim threshold was determined at 10 Hz. Test amplitude was set to below the threshold, i.e., without visual contraction of the pelvic region or awareness response, and was delivered at 10 Hz with a 1 hr. on/off duty cycle for 24 hr. once per week for several weeks. No change in normalized food or water consumption were observed across the Pre-stim, Stim, and Post-stim days. Compared to the Pre-stim day, PudS Stim significantly increased average VV and decreased the VF over a 24 hr. period during both the Stim and Post-stim days. We observed a diurnal pattern in voiding behavior during the Pre-stim condition, consisting of larger average VV with decreased VF during sleep cycles and smaller VV with increased VF during awake cycles. During the asleep state, PudS Stim did not significantly change the average VV. However, PudS Stim did significantly decrease VF on the Stim day. During the awake state, PudS Stim significantly increased average VV and decreased VF for both Stim and Post-stim days compared to Pre-stim. Our results confirm, as reported in acute animal studies, that PudS Stim is effective in changing the voiding behavior in conscious unrestrained animals. We observed significant increases in average VV and decreases in VF using a 1 hr. on/off duty cycle at 10 Hz. Furthermore, these effects persisted the following day, i.e., demonstrating a carryover effect. Our results suggest that continuous Stim may not be necessary to reduce OAB symptoms. These results provide positive evidence that electrical Stim of peripheral nerves responsible for the coordination of lower urinary tract function may have utility in treating OAB.

Disclosures: C.L. Langdale: 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. **J.A. Hokanson:** 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. **D. Degoski:** 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. **P. Milliken:** A. Employment/Salary (full or part-time):; Galvani Bioelectronics. **W.M. Grill:** 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

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.05/R15

Topic: F.07. Autonomic Regulation

Title: Regulation of insulin secretion by hypothalamic neurons

Authors: *I. PAPAZOGLOU, J.-H. LEE, Z. CUI, C. LI, M. J. KRASHES, S. RANE; NIH/NIDDK, Bethesda, MD

Abstract: Insulin is secreted from pancreatic β-cells as a response to changes in circulating nutrients and hormones. This process is tightly regulated by the autonomic nervous system that strongly innervates the pancreatic islets. Although the role of intra-islet effects of autonomic nerve terminals are well studied, little is known about the way preautonomic centers in the central nervous system regulate insulin secretion. The aim of our study is to identify the preautonomic neurons in the brain that are regulating pancreatic β-cell activity. Using a cre-dependent retrograde tracer delivered in the pancreas, we identify neuronal populations in the brain that communicate with β-cells. When chemogenetically stimulated, these hypothalamic neurons suppress insulin secretion during a hyperglycemic challenge. The same group of neurons is activated upon glycoprivic/hypoglycemic challenge. Finally, inactivation of this neuronal population leads to elevated insulin levels during fasting which results in extreme hypoglycemia. Overall, this study is revealing an insulin-regulating neuronal circuit that controls glycemic states.

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Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

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Program #/Poster #: 071.06/R16

Topic: F.07. Autonomic Regulation

Support: Natioanl Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1A6A3A1131814)

Title: Innervation of the parasympathetic nervous system visualization in human and modulation of chemogenetic activation/inhibition in mice

Authors: *C. NAMKOONG[1,2], W. SONG[3], D. CHEON[4], J. HWANG[1], H. CHOI[5,6,7];
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Abstract: The liver is innervated by both the sympathetic and the parasympathetic nervous system. Human liver parasympathetic nerves are well characterized in the neuroanatomical pattern in the human liver is unknown. In the present study, we investigated the parasympathetic innervation of human and mouse liver by passive tissue clearing method for 3D-images. We optimized passive clearing method and immunofluorescent labeling of parasympathetic neurons in the human liver and mouse tissue. In addition to visualizing of parasympathetic nerve in the liver. We performed liver passive clearing and immunohistochemistry analysis to confirm 3D-anatomical interaction of parasympathetic neurons and hepatocytes. The images show the complex and dense neuronal circuit in the liver. We next investigated the role of parasympathetic in the regulation of liver glucose metabolism by chemogenetic methods using DREADDs (designer receptors exclusively activated by designer drugs). We confirm that our chemogenetic virus and mouse model is working by electrophysiology of DMV neurons showing that CNO activates the neurons. Acute activation of neurons in the DMV region results in increasing hepatic lipogenesis and gluconeogenesis. These results suggest that specific activation/inhibition of parasympathetic neurons to might be involved in the regulation of lipid and glucose metabolism.
Key words: Liver, Parasympathetic, Chemogenetics.

Disclosures: C. Namkoong: None. **W. Song:** None. **D. Cheon:** None. **J. Hwang:** None. **H. Choi:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.07/R17

Topic: F.07. Autonomic Regulation

Title: Comprehensive overview of full thickness enteric nervous system

Authors: *D. CHEON[1], H. CHOI[2];
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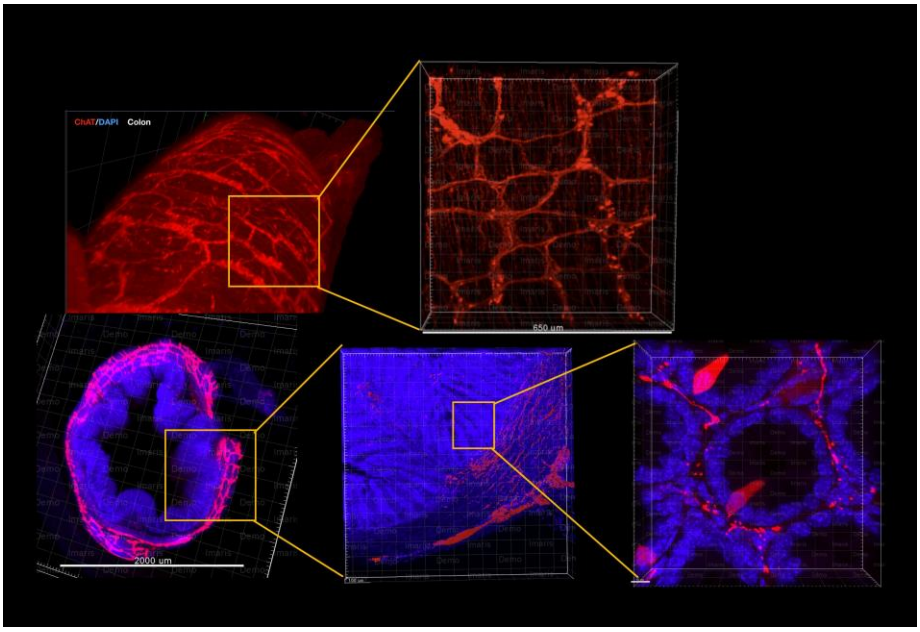
Abstract: The gastrointestinal tract is contains Mucosa, Submucosa, Muscularis Propria, Serosa. Enteric nervous system that neural network in gastrointestinal tract is known as 2parts; Submucosal Plexus and Myenteric Plexus. Myenteric Plexus is well known, especially with a number of methods of evaluation; Neuron cell body count, glia cell count, nerve type. However, Submucosal Plexus and other layers' neuronal structure is not well known yet. We investigated the cholinergic enteric nervous system structure with ChAT Cre Tomato Mouse using clearing method for 3D-images. in addition to visualizing of mesenteric nerve and method of evaluation. The images show complex and dense cholinergic neuronal structure. In this research, We discovered each layers' cholinergic enteric nerve structure in gastrointestinal tract.



Disclosures: D. Cheon: None. **H. Choi:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.08/R18

Topic: F.07. Autonomic Regulation

Support: NIH OT2OD024898

Title: Cystometrogram technique in Wistar rats impacts detrusor contractile dynamics

Authors: *D. MEDINA AGUINAGA[1], R. F. HOEY[1], M. ALTAMIRA-CAMACHO[2], A. MUNOZ[3], J. QUINTANAR-STEPHANO[2], C. H. HUBSCHER[1];
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Abstract: The profile of a nonstop trans-vesical cystometrogram (CMG) pressure curve in urethane anesthetized rats for the study of lower urinary system physiology consists of three phases: an initial increase in intravesical pressure (IVP), a plateau period with high frequency oscillations (IPHFO) accompanied by the expulsion of urine, and a rebound characterized by a further increase in IVP. The objective of the current study was to investigate the effect of catheter implantation and urethane anesthesia on the contractile pattern of the bladder during voiding. Nine female Wistar rats were used in two separate experiments. In one experiment, to observe the effect of bladder catheter implantation which necessitates urothelium injury, CMG and electromyography (EMG) of the external sphincter of the urethra was done under urethane-anesthesia with polyethylene tube (PE10) catheter placement through the left ureter into the bladder. After 10 voiding cycles, a PE60-tube catheter was implanted into the dome of the bladder and another 10 voiding cycles were recorded. In a second experiment to study the effect of urethane on the bladder, a PE50-tube catheter was placed in the dome of the bladder and tunneled under to the rat's dorsal neck. After 24 hours, a CMG on fully awake and freely moving rats was conducted, with the same rats undergoing urethane-CMG the following day for comparison. In the rats where the bladder was filled via the ureter and the urothelium remained intact, the IVP curve had a continuous ascending phase, even during expulsion of urine, without IPHFOs in the plateau phase and rebound effects. Subsequent catheterization via an incision of the vesical dome resulted in the presence of IPHFOs in the plateau phase and a rebound IVP increase. In awake CMG's 24-hours post-implantation as well as after 48-hours under urethane, contractile curves were smooth (no IPHFOs or IVP spikes). In addition, a decrease in inter-contraction interval, urine volume, and duration of the bursting phase was seen with urethane-anesthesia relative to awake conditions. Taken together, the present results demonstrate that a

lesion of the bladder wall for CMG assessments generates changes in detrusor contractile dynamics manifested as an interruption in the ascending and descending phases of bladder contraction and the presence of IPHFOs. These changes are not seen after 24 hours of post-implantation recovery and are not modified with urethane, although anesthesia has suppressive effects on the fill-void cycle.

Disclosures: D. Medina Aguinaga: None. **A. Munoz:** None. **J. Quintanar-Stephano:** None. **M. Altamira-Camacho:** None. **C.H. Hubscher:** None. **R.F. Hoey:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.09/R19

Topic: F.07. Autonomic Regulation

Title: Computational modeling of the neural circuit of rodent lower urinary tract

Authors: *B. LATIMER[1], T. BANKS[1], M. GAHL[1], V. GUNTU[1], D. J. SCHULZ[2], S. S. NAIR[3];
[1]Univ. of Missouri, Columbia, MO; [2]Div. of Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO; [3]Electrical & Computer Engin., Univ. of Missouri Columbia, Columbia, MO

Abstract: We report on findings from a biophysically constrained computational model of the rodent lower urinary tract. The effect of spinal cord injury (SCI) on this circuit is poorly understood. We combine first-hand biological recordings from pelvic ganglia neurons, a review of the literature on neurophysiology data where available, and biophysical modeling to create an *in silico* model allowing us to test hypotheses related to SCI effects on this circuit. The model includes neurons of the hypogastric ganglion, pelvic ganglion, inferior mesenteric ganglion, and spinal interneurons.
Urine storage is largely dependent on spinal reflex pathways while voluntary voiding depends on a switch from the filling state to voiding state which is thought to be mediated by top-down control from supra-spinal inputs. The model presently replicates the guarding reflex and the sympathetic storage reflex. We will use the model to investigate how normal and abnormal (e.g. detrusor-sphincter dyssynergia) functioning at the circuit level is influenced by neuronal properties and vice versa. Biological data show that neuron properties are fundamentally altered by denervation. Our model will help to explain the mechanisms underlying these effects and to investigate mechanisms that might restore the reflexive arc in SCI.

Disclosures: B. Latimer: None. **T. Banks:** None. **M. Gahl:** None. **V. Guntu:** None. **D.J. Schulz:** None. **S.S. Nair:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.10/R20

Topic: F.07. Autonomic Regulation

Support: NIH Grant DK100024

Title: The role of KCC2 in overactive bladder and central sensitization

Authors: *E. J. GONZALEZ, W. M. GRILL;
Duke Univ., Durham, NC

Abstract: Overactive bladder (OAB) is a significant unresolved health concern with considerable economic burden in lost productivity and health care. While the pathophysiology underlying OAB is unclear, central sensitization may be contributing to hypersensitivity of the bladder. The K^+/Cl^- Co-transporter 2 (KCC2) is expressed in CNS neurons to maintain the chloride concentration gradient. Loss of KCC2 function leads to depolarization of the chloride equilibrium potential and contributes to central sensitization in animal models of chronic neuropathic pain. OAB might share common pathophysiological mechanisms with neuropathic pain, and we tested the novel hypothesis that loss of KCC2 function contributes to the symptoms of OAB. The objective of this study was to determine the role of KCC2 in normal bladder storage function, and to determine whether restoration of KCC2 function can improve OAB symptoms. OAB was induced in female Wistar rats with the administration of cyclophosphamide (CYP, 150 mg/kg IP), and acute experiments were conducted under urethane anesthesia (1.2 g/kg SQ, supplemented as needed) 48 hours after injection. The bladder was exposed through a midline abdominal incision and a catheter was inserted into the bladder dome and electrodes were placed on the external urethral sphincter (EUS) for single-trial cystometry with EUS EMG. Rats treated with CYP demonstrated reductions in bladder capacity (1.5 fold), as well as increases in the number of non-voiding contractions compared to naive rats (1.7 fold). We then measured the effects on bladder filling of intrathecal (L6-S1) administration of the selective KCC2 inhibitor, VU0240551 (40-80µM), the selective KCC2 blocker, DIOA (3.75mM), and the KCC2 extrusion enhancer, CLP257 (100mM). Inhibition of KCC2 function with VU0240551 produced reversible reductions in bladder capacity of naive rats (1.4 fold), whereas DIOA had no effect on bladder capacity. Enhancement of KCC2 function with CLP257 produced reversible increases in bladder capacity of rats treated with CYP (1.4 fold). These studies suggest a role for KCC2 dysfunction in the storage symptoms of OAB, and identify a novel therapeutic approach to improve bladder capacity.

Disclosures: E.J. Gonzalez: None. **W.M. Grill:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.11/S1

Topic: F.07. Autonomic Regulation

Support: Wallace H. Coulter Foundation

Title: Age related reduction of urethral afferents sensitivity

Authors: *A. GERAMIPOUR[1], Z. C. DANZIGER[2];
[2]Biomed. Engin., [1]Florida Intl. Univ., Miami, FL

Abstract: Age-related underactive bladder (UAB) can lead to high healthcare expenses, lower urinary tract (LUT) infection and even death in elderly population, yet the underlying causes of age-related UAB are still unknown. Complete bladder emptying requires the activation of voiding reflexes, and intact urethral afferent signaling is crucial to the normal function of urinary tract reflexes. The augmenting reflex is the activation of a reflex from the urethral afferents to the bladder efferent, which leads to a bladder contraction, and failure of this reflex disrupts the efficient voiding. Our previous work showed that the augmenting reflex is functionally impaired in older animals; in this study, we measure directly the urethral afferents to investigate if urethral signaling weakens with age and if this loss of sensitivity drives reduced reflex function. We used a range of urethane-anesthetized female Sprague-Dawley rats across their natural lifespan to quantify the effect of aging on the urethral afferents signaling. A catheter was passed through the intravesical space into the urethra through the bladder dome to allow infusion of fluid through the urethra. The abdomen was sutured closed but we left the bladder incision open so that the bladder remained empty during the experiment. The pudendal nerve was exposed using the posterior approach and the sensory branch was isolated from the compound nerve and connective tissue. A bipolar nerve cuff electrode was placed on the sensory branch of the pudendal nerve and at 2-minute intervals the urethra was infused at a pseudorandomly selected flow rates to compare the urethral afferents response to a range of urethra flow rates in different animal age groups. Preliminary results show that urethral afferent signaling is weaker in older animals. The sensitivity of urethral afferents to urethral flow was shifted to higher flow rates with age, indicating that higher flow rates are required in older animals to recruit the urethral afferents. Therefore, the signaling reduction of urethral afferents to flow may weaken the augmenting reflex activation and contribute to incomplete bladder emptying in elderly population. This experiment enables us to test the function of urethral afferents to understand how aging impairs voiding efficiency.

Disclosures: A. Geramipour: None. **Z.C. Danziger:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.12/S2

Topic: F.07. Autonomic Regulation

Support: NIH OT2-OD023867
NIH R21-DK116029
NIH R21-EB024701

Title: Respiratory-gated transcutaneous vagus nerve stimulation increases 4D cine MRI-assessed stomach emptying in functional dyspepsia

Authors: *R. SCLOCCO[1], C. NGUYEN[1], R. STALEY[1], H. FISHER[1], C. VELEZ[1], A. MENDEZ[1], K.-H. LU[2], Z. LIU[2], M. WARD[2], T. L. POWLEY[2], N. W. KETTNER[3], B. KUO[1], V. NAPADOW[1];
[1]Massachusetts Gen. Hospital, Harvard Med. Sc, Boston, MA; [2]Purdue Univ., W Lafayette, IN; [3]Radiology, Logan Univ., Chesterfield, MO

Abstract: The vagus nerve plays an important role in both sensory and motor aspects of gastric physiology. Both animal and human studies suggest that accommodation reflex consists of a vagovagal reflex pathway. Thus, modulating vagal efference may be promising to increase gastric hypomotility for gastroparesis or functional dyspepsia.
Transcutaneous vagus nerve stimulation (tVNS) of the auricular branch of the vagus nerve is a promising non-invasive neuromodulatory therapy for numerous disorders. We have demonstrated that tVNS targets nucleus tractus solitarii, and medullary efferent parasympathetic (vagal) nuclei (Sclocco et al. 2019). Our group showed that neural target-engagement can be enhanced by gating stimulation to the exhalation phase of respiration, via Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS).
In this ongoing study, we evaluate twelve (12) healthy controls and five (5) functional dyspepsia (FD) patients with both brain fMRI and 4D cine MRI of the stomach during a test meal challenge and concurrent RAVANS or Sham tVNS, to investigate effects on gastric emptying. MRI examinations were performed immediately following ingestion of a food-based MRI contrast test meal (470ml, pineapple-based with high manganese content), using 4D cine MRI (3D stack of stars GRE FLASH sequence, temporal resolution = 7s, FA = 3deg, 40 slices, 2.1x2.1x3.5mm voxel size, 40 volumes collected). Each subject was scanned 15, 65, and 80 minutes post-meal (T0, T1, T2), while experiencing active RAVANS (300μs pulse width, 1.5s stimulation trains delivered at 100Hz in the cymba concha of the left ear) or Sham (no current) on two different visits (randomized order). Abdominal MRI images were segmented to isolate the stomach, meal

and air contents (semi-automated, based on image contrast) for each subject and post-meal time point, and percent volume changes from T0 were calculated for T1 and T2.
In FD patients, percent reduction in meal volumes during RAVANS were significantly higher compared to Sham from T0 to T1 (RAVANS: -22.80 ± 12.36%, mean ± SD; Sham: -13.83 ± 5.52%; p-val = 0.037), and approaching significance from T0 to T2 (RAVANS: -36.92 ± 10.62%, mean ± SD; Sham: -28.33 ± 17.00%; p-val = 0.089). No significant differences in percent volumetric reductions between RAVANS and Sham sessions were found in the control group.
Although preliminary, these results support the hypothesis that RAVANS can successfully modulate gastric motility in FD patients. Future investigations will link fMRI response to RAVANS with gut motility to investigate the brain-gut axis underlying tVNS-modulated gastric physiology.

Disclosures: **R. Sclocco:** None. **C. Nguyen:** None. **R. Staley:** None. **H. Fisher:** None. **C. Velez:** None. **A. Mendez:** None. **K. Lu:** None. **Z. Liu:** None. **M. Ward:** None. **T.L. Powley:** None. **N.W. Kettner:** None. **B. Kuo:** None. **V. Napadow:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.13/S3

Topic: F.07. Autonomic Regulation

Support: R01DK084060

Title: Acid sensing ion channel 3 controls afferent sensitization in a model of cystitis induced by cyclophosphamide

Authors: N. MONTALBETTI, J. G. ROONEY, ***M. D. CARATTINO**;
Med., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Acid-sensing ion channels (ASICs) are heterotrimeric proton-gated cation selective ion channels that play roles in mechanoreception and nociception in the peripheral nervous system. In this report, we investigated the contribution of ASIC3 to nociception in a model of cystitis induced by cyclophosphamide (CYP). To induce cystitis, mice were administered CYP (80 mg/kg) every other day for up to a week and experimental procedures were conducted a day after the last dose. In wild type (WT) mice, a significant reduction in the mechanical threshold to von Frey filaments applied to the pelvic area was observed after 4 doses of CYP (8 days). Strikingly, male and female ASIC3 KO mice treated with CYP exhibited an exaggerated response to von Frey filaments applied to the pelvic area 6 days after the initial dose of CYP, a

time point at which only modest changes in pelvic sensitivity were apparent in WT mice. Consistent with these results, the visceromotor response to bladder distension was greater in female ASIC3 KO mice treated with CYP than saline or WT treated with CYP. To determine whether the enhanced pelvic sensitivity seen in ASIC3 KO mice treated with CYP reflects changes in sensory neuron excitability, we conducted patch-clamp analysis to examine the firing rate of acutely isolated bladder sensory neurons from ASIC3 KO mice and WT littermates injected with saline or CYP (6 days). Bladder sensory neurons were classified on the basis of the sensitivity of the action potential to 1 μM tetrotodoxin (TTX). Bladder sensory neurons with TTX-sensitive (TTX-S) action potentials from WT and ASIC3 KO mice treated with CYP discharged more action potentials in response to electrical stimulation than controls that received saline. Strikingly, our studies revealed that CYP treatment sensitized bladder sensory neurons with TTX-resistant (TTX-R) action potentials (C fibers) in ASIC3 KO mice, but not in WT littermates. These results indicate that ASIC3 regulates the process of afferent sensitization, and that the differences in pelvic sensitivity between WT and ASIC3 KO mice treated with CYP are mediated at least in part by sensitized C fibers.

Disclosures: M.D. Carattino: None. **N. Montalbetti:** None. **J.G. Rooney:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.14/S4

Topic: F.07. Autonomic Regulation

Support: NIH RO1DK117404
CVRI Collaborative Research Grant

Title: Understanding the role of free fatty acid receptor 3 (FFAR3) in mediating gut-brain communication in obesity

Authors: *T. COOK[1], R. BONOMO[1], C. GAVINI[1], L. GAUTRON[2], B. LAYDEN[3], V. MANSUY-AUBERT[1];
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Abstract: More than 100 million Americans are obese, representing over 35% of the Unites States population. Western diet (WD) is a major cause of the obesity epidemic increasing prevalence of many other comorbidities, particularly type 2 diabetes. The western diet consisting of high saturated fat, high cholesterol, high sugar, and heavily processed foods robustly disrupts gut bacteria composition. The microbiome ferments non-digestible fibers releasing short chain

fatty acids (SCFA's) which serve as energy substrates as well as signaling molecules via free fatty acid receptors 2 (FFAR2) and 3 (FFAR3). Gut microbiome dysregulation is associated with changes in SCFA levels that correlate with obesity in humans and mice. The SCFA's propionate and butyrate bind FFAR3 with high affinity, and FFAR3 signaling modulates energy expenditure via sympathetic nervous system activation. The direct role FFAR3 in gut-brain communication via the vagus nerve is currently poorly understood. The vagus primarily consists of sensory afferents which relay specific nutrient information to the brain. Several studies have shown that vagus nerve disruption eliminates effects of the microbiome and SCFA's altering metabolism, yet the molecular mechanisms remain largely unknown. We have found that western diet altered SCFA profiles and reduced FFAR3 expression in the nodose ganglia. We confirmed previous findings that fecal microbiome transplantation (FMT) from lean mice reversed some WD-induced changes to bacterial composition, plasma SCFA's, and glucose homeostasis. Our data suggest that altered FFAR3 signaling in vagal afferent neurons may contribute to some of the metabolic changes seen upon western diet feeding, and that gut microbes transplanted from lean to obese mice may alter gut brain communication through SCFA's binding FFAR3 directly on the vagus nerve.

Disclosures: **T. Cook:** None. **R. Bonomo:** None. **C. Gavini:** None. **L. Gautron:** None. **B. Layden:** None. **V. Mansuy-Aubert:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.15/S5

Topic: F.07. Autonomic Regulation

Support: PAPIIT-UNAM IA203617 (FC)
CONACyT scholarships 781480 (SYRJ)
CONACyT scholarship 861149 (GCHH)

Title: The expression of the G protein-coupled estrogen receptor (GPR30) in pelvic floor muscles seems unrelated to serum estradiol levels in female rabbits

Authors: S. Y. RODRÍGUEZ-JAIMES[1], G. C. HERNÁNDEZ-HERNÁNDEZ[2], A. CARRASCO-RUIZ[3], M. MARTINEZ-GOMEZ[4], E. CUEVAS[5], *F. CASTELÁN[6];
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Abstract: Pelvic floor muscles (PFM) assist importantly in micturition. Its weakening is related to the appearance of gynecological and urinary dysfunctions in women, showing a higher prevalence at menopause. Hence, the role of estrogens has received much attention to understand the physiopathology of pelvic organ prolapses and stress urinary incontinence. Our present study aimed therefore to evaluate the relative expression of the G protein-coupled estrogen receptor (GPR30) in bulbospongiosus and pubococcygeus muscles, and its possible association with serum estradiol levels. We used female rabbits randomly allocated in control (C), 1-month ovariectomized (OVX), and OVX plus estradiol benzoate (OVX+EB) groups. For this sake, ELISA, Western blot, and immunohistochemistry were used. Serum estradiol levels were significantly decreased in the OVX group and significantly increased in the OVX+EB group. We identified the GPR30 expression in pubococcygeus and bulbospongiosus muscles, but this was independent of estradiol levels. Both muscles showed a GPR30 immunoreactivity next to myonuclei and in apparent polymorphonuclear cells. Together, these results suggest that serum estradiol levels are unrelated to GPR30 expression in bulbospongiosus and pubococcygeus muscles of female rabbits. Our findings also imply that further hypothesis about estrogen modulation of PFM should consider rapid estrogen actions.

Disclosures: S.Y. Rodríguez-Jaimes: None. **G.C. Hernández-Hernández:** None. **A. Carrasco-Ruiz:** None. **F. Castelán:** None. **E. Cuevas:** None. **M. Martinez-Gomez:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.16/S6

Topic: F.07. Autonomic Regulation

Support: CONACYT NMA 591688
CONACYT BECA MIXTA NACIONAL NMA 291211
SEP-DEGESU

Title: Sensory and postganglionic neurons of the urethra in female rats

Authors: *N. MIRTO AGUILAR[1], A. D. DIAZ[2], C. MORAN[3], Y. CRUZ[4];
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Abstract: Lower urinary tract (LUT) comprises bladder and urethra. Afferent and efferent innervation of the LUT arises in the lumbosacral and thoracolumbar spinal cord. The fibers are

carried in three sets of nerves: pelvic, pudendal and hypogastric. Although there are plenty of information sensory and postganglionic neurons of the bladder, little is known about the innervation of the urethra. The autonomic innervation is mostly provided by the pelvic plexus. The aim of the present study was to determine the number of sensory and postganglionic neurons of the urethra in virgin female rat. Eleven adult virgin Wistar female rats were used. 5 µl of True Blue was injected in three regions of the urethra; pre-pelvic, pelvic and perineal (n=4-3 per region). After seven days of survival, the rats were anesthetized with sodium pentobarbital and transcardially perfused with saline solution followed by a fixative consisting of 10% of formalin. Then, the dorsal root ganglia T13-S2 (DRG) and the major pelvic ganglion (MPG) were removed, post-fixed for 24-48 h, placed in PBS containing increasing concentrations of sucrose (10%, 20% and 30%, 24 hours in each concentration) at 4°C and longitudinally sectioned (14 µm) on cryostat. The slides were examined with a fluorescence microscope, and the cells labeled with True Blue were counted at a magnification of 10X using AxioVision Rel 4.6 software (Zeiss Software Inc). Only labeled cells with a clearly visible nucleus were counted. Cell counts were corrected by the method of Abercrombie. We found positive true blue sensory cells from T13 to S2 DRG. The neurons were located throughout the dorsal root ganglia and presented a somatotopic organization. Most of the sensory neurons of the pre-pelvic and pelvic regions of the urethra were found in L6 (36.6%; 41.5%), S1 (27.5%; 31.4%), L2 (12.6%; 8.3%) and L1 (12.2%; 8.9%). Labeled neurons of the perineal region of the urethra were found in L6 (82.9%) and S1 (13.9%). The true blue labeled postganglionic neurons were localized in the caudo-medial region of the MPG, with ~500 neurons innervating each region (pre-pelvic, 421±14 neurons; pelvic, 555±9 neurons and perineal 528±6 neurons). In conclusion, there is a regionalized organization of the sensory and postganglionic neurons of the urethra.

Disclosures: **N. Mirto aguilar:** None. **A.D. Diaz:** None. **C. Moran:** None. **Y. Cruz:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.17/S7

Topic: F.07. Autonomic Regulation

Support: NIH K12GM111725
NIH R01DK068400

Title: Pomc-deficient mice have altered reproductive function

Authors: *Z. THOMPSON[1], O. ELEGBEDE[4], M. MEYERS[5], G. L. JONES[2], J. M. ADAMS[3], H. YU[1], M. J. LOW[1];
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Abstract: The pro-opiomelanocortin (*Pomc*) gene encodes POMC, which is differentially processed to produce adrenocorticotrophin, beta-endorphin, and three melanocyte-stimulating hormones, among other peptides. POMC neurons are principally located in the arcuate nucleus (Arc) of the hypothalamus, where they are essential in the control of food intake, energy expenditure and body weight. Several different mutations in the *POMC* gene have been shown to cause early-onset obesity and adrenal cortical insufficiency in humans.
Two 5' distal enhancers regulate neuron-specific *Pomc* expression in the Arc. Insertion of a LoxP flanked neomycin resistance cassette combined with deletion of both enhancer sequences selectively reduces the amount of *Pomc* mRNA and POMC peptides in Arc neurons, but not pituitary cells. These mice (ArcPOMC$^{-/-}$) have less than one percent of Arc *Pomc* mRNA compared to wildtype mice. Like other genetic mouse models of obesity, ArcPOMC$^{-/-}$ mice are infertile, but it is unclear whether their reproductive disruption is due primarily to POMC-deficiency in the brain or is secondary to obesity.
We compared aspects of reproductive function in wildtype (ArcPOMC$^{+/+}$), heterozygous (ArcPOMC$^{+/-}$) and ArcPOMC$^{-/-}$ female mice (n = 10, 17, 10, respectively). Investigators were blinded to genotype during data collection and analysis. There were no significant differences in day of vaginal opening or day of first estrus among the groups. However, the number of estrus cycles in 4 weeks was significantly different among the three groups, and ArcPOMC$^{-/-}$ mice had significantly fewer estrus cycles (one-way ANOVA $p = 0.0024$). A Dunnett's multiple comparisons test for differences between groups gives a corrected p-value of $p = 0.0031$ for ArcPOMC$^{+/+}$ vs. ArcPOMC$^{-/-}$, and $p = 0.8041$ for ArcPOMC$^{+/+}$ vs. ArcPOMC$^{+/-}$.
In addition, we are using a related, conditional mutant mouse model (FNeoΔ2) in which *Pomc* gene expression can be restored temporally by deletion of the neomycin resistance cassette using a tamoxifen-inducible Cre-ERT2 transgene. Because women with mutations in the *POMC* gene also experience disruptions in timing of puberty, or a cessation of pubertal development, understanding more about how hypothalamic POMC-deficiency impacts reproduction in mice may help to develop therapies for humans impacted by similar mutations.

Disclosures: Z. Thompson: None. **O. Elegbede:** None. **M. Meyers:** None. **G.L. Jones:** None. **J.M. Adams:** None. **H. Yu:** None. **M.J. Low:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.18/S8

Topic: F.07. Autonomic Regulation

Support: NIH Grant 2R44NS065545-03A1
NIH SPARC OT2 OD023847

Title: Closing the loop on gastric electrical stimulation: Multichannel recording platform for non-invasive measurement of vagal nerve activity

Authors: M. P. WARD[1], T. NOWAK[3], A. GUPTA[4], T. L. POWLEY[2], J. FURNESS[5], M. SONNTAG[6], *A. HARRISON[6], E. BROWN[6], A. M. MELEHAN[7], M. ISAF[7], I. P. CLEMENTS[6];
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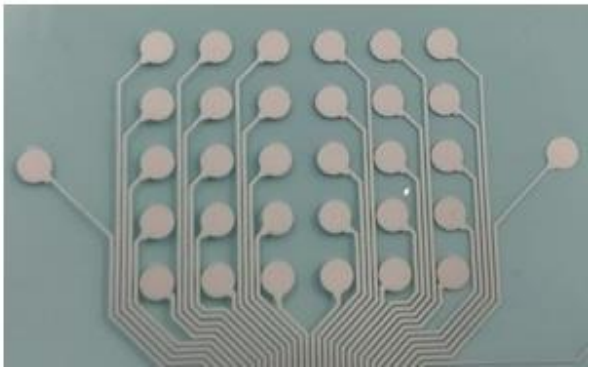
Abstract: Gastroparesis (GP) is a chronic condition characterized by a slowed transit of food from the stomach without any visible obstruction. When dietary modification and medications fail, gastroparetic patients may become candidates for gastric electrical stimulation (GES) therapy, a potentially life changing neurostimulation therapy for intractable forms of nausea and vomiting associated with diabetic or idiopathic GP. Despite common reports of symptom improvement from open-label studies using the Medtronic Enterra GES device, the time-to-efficacy and degree of symptom relief are unpredictable. Variable reports of efficacy may stem from improper stimulating electrode placement, the open-loop, “one-size-fits-all” stimulus parameter tuning protocol, an incomplete understanding of the mechanisms-of-action, or a general lack of unified knowledge of the underlying anatomy and functions of the vagal-gut connectome. Prior research has identified vagal activity associated with efficacy of GES, and proposed noninvasive investigation of vagal activity for clinical applications. Specifically, novel nerve response analysis techniques (Autonomous Neural Control, or ANC) and machine learning techniques were used to associate specific vagal activity and efficacy of GES. Preliminary results correlated specific vagal activity patterns with alleviation of specific symptoms [1]. Here we describe development and use of a new multichannel recording platform for sensitive and high-resolution measurement of underlying nerve activity. This system is being tuned towards enhanced characterization of vagal nerve activity. Ultimately, the investigators hope that clinicians may use this technology during GES device implantation and optimization to better determine device placement and stimulation parameters.
[1] M. P. Ward, K. Y. Qing, R. M. Worth *et al.*, “A flexible platform for biofeedback-driven control and personalization of electrical nerve stimulation therapy,” *IEEE TNSRE,* vol. 23, no. 3, pp. 475-484, 2015.



Disclosures: **M.P. Ward:** None. **T. Nowak:** None. **A. Gupta:** None. **T.L. Powley:** None. **J. Furness:** None. **M. Sonntag:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioCircuit Technologies. **A. Harrison:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioCircuit Technologies. **E. Brown:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioCircuit Technologies. **A.M. Melehan:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **M. Isaf:** None. **I.P. Clements:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioCircuit Technologies.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.19/S9

Topic: F.07. Autonomic Regulation

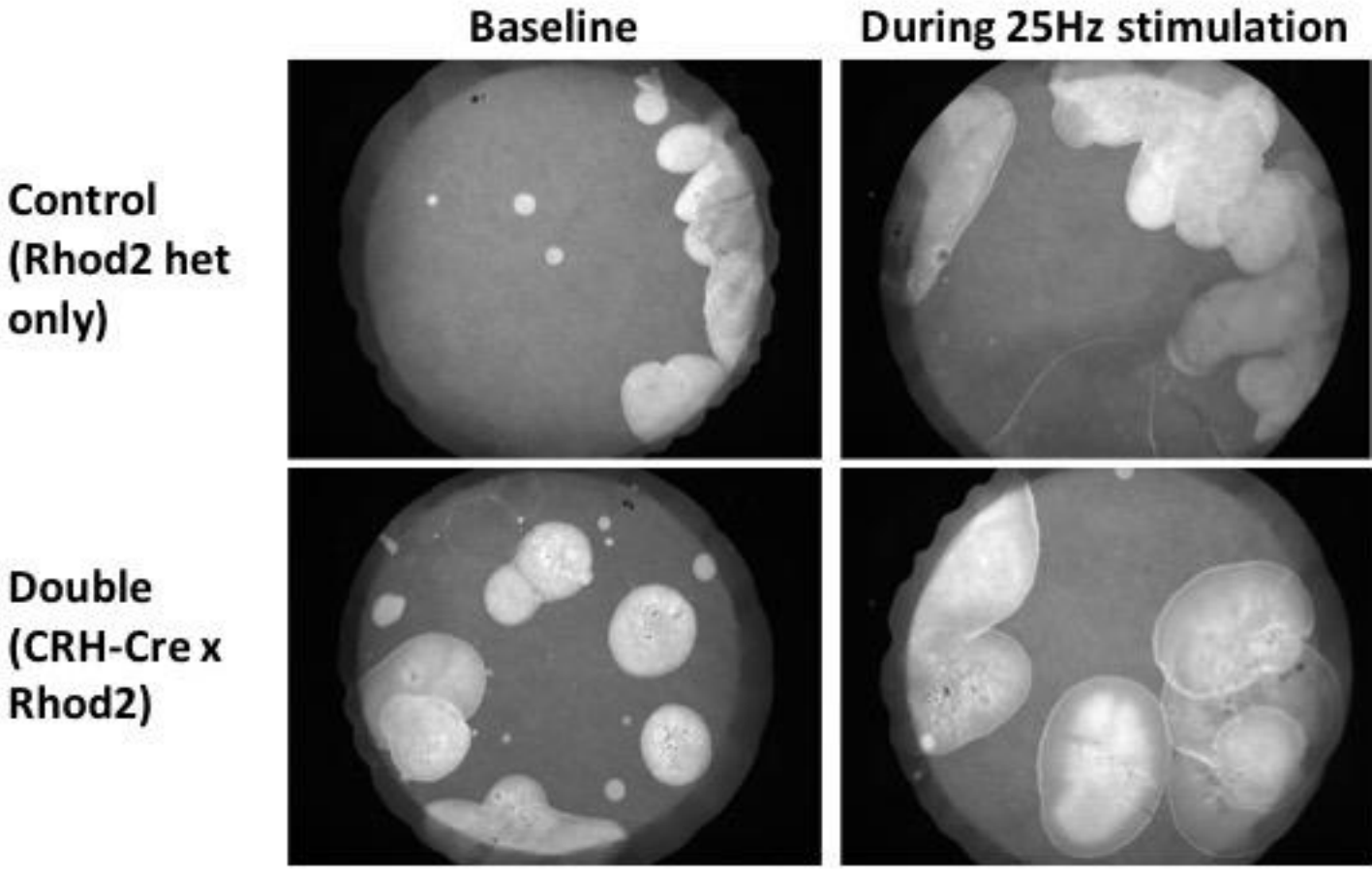
Support: Urology Care Foundation Research Scholar Award Program
NCATS/NIH KL2TR001879

Title: Long-term optogenetic stimulation of CRH specific neurons in Barrington's nucleus as a model of bladder hypertrophy

Authors: *J. P. VAN BATAVIA[1], S. BUTLER[1], J. FESI[1], S. VICINI[2], S. ZDERIC[1];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Dept Parmacology & Physiol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Symptoms of lower urinary tract dysfunction (LUTD) affect 20% of “normal” children. A common LUTD is voiding postponement in which children void infrequently and with large volumes. This condition is modeled in mice subjected to social stress who show decreased voiding frequency and increased voided volumes along with increases in corticotropin-releasing hormone (CRH) expression in Barrington’s nucleus (BN), the pontine micturition center. We have previously shown that activation of these neurons using optogenetics leads to larger bladder volumes and longer time periods between voids in the acute setting. Here we examined the effects of optogenetic stimulation of CRH BN neurons on the *in vivo* voiding phenotype and bladder mass in awake mice during long-term stimulation. Control and double transgenic mice expressing channel 2 rhodopsin (Chr2) in CRH cells had fiberoptic probes implanted into BN at 8 weeks of age (n =4 for each). *In vivo* voiding pattern (number of voids/24 hr period and volume per void) were obtained before and during 2 weeks of optogenetic stimulation 25 Hz (15msec pulses, 5 seconds on, repeated every minute) (see Figure 1). During optogenetic stimulation in the double transgenic mice, the voiding pattern changed from a mean of 13 voids per day at baseline to 3.5 voids per day at the 2-week mark. Similarly, in the double transgenic mice, the mean voided areas increased by 370% between baseline and 2 week mark during optogenetic stimulation. There were no differences in either parameter in the control mice. Furthermore, bladder weight as a ratio of total body weight was increased in the double transgenic mice compared to controls after 2 weeks of stimulation (1.5 vs. 1.05, $p < 0.05$). Our results suggest that long term high frequency optogenetic stimulation of CRH-BN neurons produces a sustained change in voiding pattern that mimics the social stress voiding pattern of infrequent, larger voids. After 2 weeks of stimulation, bladder weight also increased in

stimulated mice suggesting that optogenetic stimulation of CRH BN neurons may be a reproducible model for bladder hypertrophy.



Voiding blot patterns in control mouse (heterozygous for Rhod2 gene only; top row) compared to double transgenic CRH-Cre x Rhod2 mouse (bottom row) at baseline (prior to optogenetic stimulation; first column) and 24 hours after continuous stimulation with 470nm light at 25Hz (second column).

Disclosures: **J.P. Van Batavia:** None. **S. Butler:** None. **J. Fesi:** None. **S. Vicini:** None. **S. Zderic:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.20/S10

Topic: F.07. Autonomic Regulation

Support: NIH DK106456
NIH DK084567
NIH DK100223
AGA RSA

Title: Sensory epithelial gastrointestinal enteroendocrine cells use different signaling pathways for chemo and mechanotransduction

Authors: *C. ALCAINO[1], K. KNUTSON[1], S. T. WHITEMAN[1], V. NAYAK[1], H. KACMAZ[1], A. J. TREICHEL[1], P. R. STREGE[1], J. H. LI[2], A. B. LEITER[2], G. FARRUGIA[1], A. BEYDER[1]; [1]Mayo Clin., Rochester, MN; [2]Univ. of Massachusetts, Worcester, MA

Abstract: Background: The enteroendocrine cells (EECs) in the gastrointestinal (GI) epithelium are important specialized sensors of luminal forces and chemicals, like nutrients and bacterial metabolites. EECs regulate GI and systemic physiology by releasing a range of important signaling molecules, like serotonin and incretins. EEC signal transduction mechanisms are important but remain poorly understood. EEC chemotransduction depends on the voltage-gated Ca^{2+} channels (Cavs) L-, P/Q-, but not T-type. T-type Cavs are highly expressed in EECs and involved in mechanotransduction in other specialized mechanosensors, but their role in EECs is unknown. Whether EEC mechanotransduction follows a differential pathway remains to be clarified. **Aim**: To determine the role of T-type Cavs in EEC mechanotransduction. **Methods**: We lineage-traced EECs using the transcription factor NeuroD1, which is expressed selectively by late EEC precursors. We created two mouse models by breeding *NeuroD1-cre* with RiboTag and tdTomato/GCaMP5 mice. We bred *Cav3.2-Cre* mouse with tdTomato to track T-type Cav3.2 epithelial expression. We evaluated EEC Cav expression by RT-qPCR and IHC and function in primary cultures by whole cell voltage-clamp and by Ca^{2+} imaging with either membrane displacement or the Trpa1 channel agonist AITC. **Results**: Purified NeuroD1 EEC transcripts were enriched for epithelium markers (*Vil1*), EECs (*ChgA*), and Cavs: L- (*Cacna1a*), P/Q- (*Cacna1d*) and T-type (*Cacna1h*) (n=3, $p<0.05$). IHC showed Cav3.2 specifically within NeuroD1 EECs and ChgA in Cav3.2+ cells. Electrophysiology revealed a rapidly activating and inactivating voltage-dependent Ba^{2+} current that was blocked by T-type Cav blocker mibefradil. Both mechanical ($\Delta F/F0$ 1.6±0.6) and chemical stimulation with AITC ($\Delta F/F0$ 1.7±0.3) induced intracellular Ca^{2+} increase. Mechanosensitive responses were inhibited by Ca^{2+} substitution (-

96±1% Ca^{2+} free), non-selective Ca_V block (-71±9% nickel), T-type Ca_V block (-61±6% mibefradil), and P/Q-type Ca_V block (-80±4% ω-agatoxin IVA), but not L-type Ca_V block (2±5% nifedipine) (n=3-18, $p<0.05$, except nifedipine vs control). $Ca_V3.2$ knockdown blocked mechanosensitive ($\Delta F/F0$ 0.5±0.3), but not chemosensitive responses (1.5±0.4 AITC), suggesting $Ca_V3.2$ is specifically required for mechanotransduction. **Conclusions**: GI epithelium EECs specifically express Ca_V isoforms. EEC mechanotransduction requires T- and P/Q-, but not L-type Ca_Vs. $Ca_V3.2$ knockdown specifically blocks mechano, but not chemosensitivity, suggesting EECs utilize different signaling pathways for chemo and mechanotransduction.
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Disclosures: C. Alcaino: None. **K. Knutson:** None. **S.T. Whiteman:** None. **V. Nayak:** None. **H. Kacmaz:** None. **A.J. Treichel:** None. **J.H. Li:** None. **A.B. Leiter:** None. **G. Farrugia:** None. **A. Beyder:** None. **P.R. Strege:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.21/S11

Topic: F.07. Autonomic Regulation

Support: Conquer Paralysis Now
International Foundation Research in Paraplegia
Brocolli Foundation

Title: Transcutaneous electrical spinal cord neuromodulation (TESCoN) improves bladder and bowel function after neurological dysfunction

Authors: *P. GAD[1], K. LATACK[2], E. KREYDIN[2], H. ZHONG[3], V. EDGERTON[4];
[1]Univ. Of California Los Angeles, Los Angeles, CA; [2]Univ. of Souther California, Los Angeles, CA; [3]Integrative Biol. and Physiol., UCLA, Los Angeles, CA; [4]Dept Integrative Biol. & Physiol., Univ. of California Los Angeles, Los Angeles, CA

Abstract:
Bladder and bowel dysfunction impacting patient's quality of life is universal after neurological disorder. Current therapies focus on managing symptoms and complications rather than restoring function. It is generally thought that voluntary bladder control resides in the cortex/brainstem. However, we have demonstrated, presence of functionally significant levels of bladder control derived from neural centers in the lumbosacral spinal cord. Thus, this study investigates use of transcutaneous electrical spinal cord neuromodulation (TESCoN) to activate the bladder and bowel and to improve function. Twelve patients (6 SCI, 4 Stroke and 2 MS) were recruited for

this study. Urodynamic assessments were performed without and with TESCoN. All patients received TESCoN therapy (T11 and L1). Validated bladder and bowel questionnaires, urodynamic properties and bladder diaries pre and post therapy were performed. Acutely, TESCoN increased voiding efficiency and enabled volitional voiding with increased sensation. After the 8-week therapy, all patients demonstrated an increased bladder capacity, decreased number of incontinence episodes and urge, improvement in questionnaire scores and an increased sensation of bladder fullness. Some (n=8) patients reported improvements in bowel movements with lower time needed to complete, reduced reliance on digit-stimulation/suppository, increased frequency of volitional bowel movements per week with no fecal incontinence episodes. Better sensation of bladder fullness and increased capacity allowed the SCI patients to time catheterization efficiently and safely. The ability to volitionally void with sensation during TESCoN gave SCI patients an increased independence and satisfaction. Reduced urge and incontinence allowed stroke and MS patients to void at their convenience. Despite the varied pathology all patients responded similarly to TESCoN demonstrating the presence of spinal neural control of bladder and bowel in humans. No adverse events were observed.

Disclosures: **P. Gad:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); spineX inc.. **K. Latack:** None. **E. Kreydin:** None. **H. Zhong:** None. **V. Edgerton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); spineX inc..

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.22/S12

Topic: F.07. Autonomic Regulation

Support: FONDECYT: 1140776 to JAB
FONDECYT: 1190729 to JAB
FONDECYT: 1191041 to WQ
FONDECYT: 1181019 to MJP
IDRC

Title: Effects of arsenic exposure on blood brain barrier and colonic permeability in healthy young rats

Authors: *C. A. BARRERA BUGUEÑO, I. HERESMANN, W. QUIROZ, M. JULIO-PIEPER, J. A. BRAVO;
Inst. de Quimica, Pontificia Univ. Catolica De Valparaiso, Valparaiso, Chile

Abstract: Arsenic (As) is a toxic metalloid, which has become a global health burden as millions of people are exposed to different sources. Growing evidence indicates that As has harmful effects on the central nervous system (CNS), as this metalloid crosses the blood-brain barrier (BBB). The BBB regulates the contact between CNS and circulating elements, so an increase in BBB permeability has generally been associated with CNS inflammation and diseases. On the other hand, there is little evidence that suggests that loss of intestinal permeability might affect BBB permeability, thus allowing environmental contaminants, such as As to permeate the CNS. The aim of this study is to evaluate if oral exposure to As affects intestinal and BBB permeability, as this pollutant might have an impact on what now is known as the brain-gut axis. Methods: Female Sprague-Dawley rats (PND35) where given 10 ppm of $NaAsO_2$ in the drinking water for 24h (n=5), and compared to control rats (n=6). General health status was monitored for each animal. Water consumption was measured. At 24h the following samples were collected: brain, colon, lung, stool and liver. Each sample was lyophilized and then microwave digested in order to determinate total As concentration. The analyte was determined by HPLC-HG-AFS. Additionally, colonic permeability to FITC-dextran 4.4kDa (FD4) was evaluated *ex vivo* for 120 and 180 min by everted gut sac technique. Results: There is no overall effect of 24h exposure to As in the animals general health status, while water consumption between groups was similar. Gut permeability to FD4 is increased in animals exposed for 24h to 10 ppm of $NaAsO_2$ in comparison to controls. In addition, the metalloid concentration was higher in every studied tissue of exposed rats, in comparison to controls. In the brain, As was found in hypothalamus and in cerebral cortex. The latter finding suggest that As is able to cross the BBB. These data indicate that As exposure in the drinking water increases gut permeability in the rat, an effect that might lead to alterations in BBB, thus allowing the passage of As into the CNS. In conclusion, a toxic pollutant such as As might cause alterations in the brain-gut axis, effects which gives a novel approach in the study of As toxicity.

Disclosures: C.A. Barrera Bugueño: None. **I. Heresmann:** None. **W. Quiroz:** None. **M. Julio-Pieper:** None. **J.A. Bravo:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.23/S13

Topic: F.07. Autonomic Regulation

Support: Fondecyt 1181019
PUCV-DI 125.726/2018

Title: Juvenile rats exposed to high fat diet display rapid changes in colon submucosal neurons

Authors: *M. JULIO-PIEPER[1], F. VILLALOBOS-MANRÍQUEZ[1], M. ZAMORANO-CATALDO[1], A. LÓPEZ[1], N. CÁCERES[1], J. EYZAGUIRRE-VELÁSQUEZ[1], J. ESCOBAR-LUNA[1], J. A. BRAVO[1], G. CRUZ[2];
[1]Grupo de NeuroGastroBioquímica, Inst. de Química, Pontificia Univ. Catolica De Valparaiso, Valparaiso, Chile; [2]Lab. de Alteraciones Reproductivas y Metabólicas, Ctr. de Neurobiología y Plasticidad Cereb, Univ. de Valparaíso, Valparaiso, Chile

Abstract: Diet composition is key to several aspects of gastrointestinal physiology, including enteric nervous system (ENS) function. In response to environmental stimuli, the ENS modulates muscle contractility and epithelial absorption/secretion with remarkable plasticity, including changes in neuron count, size and chemical coding. We have shown that, after just 20 days of protein malnutrition, juvenile rats display a reduced body size in various submucosal neuron types, including VIPergic and NPYergic neurons, in comparison to age-matched controls. Although high fat (HF) diet can also alter the size and number of certain myenteric neuronal types, little is known regarding HF diet effects on submucosal neurons. This part of the ENS is located closer to the gut lumen, and is considered a key element to the gut-brain axis. Our aim was to evaluate whether HF diet alters submucosal neuron plasticity in the juvenile rat colon. After weaning at postnatal day (PND) 21, male rats received control diet (14% energy from fat) until PND 30. Thereafter, they were separated into control groups, which continued under the same diet, and HF groups, which were fed a high fat diet (62% energy from fat). Colon barrier function analyses, submucosal neuron morphometry and characterization of chemical coding (Substance P (SP), Somatostatin, VIP and NPY) were made at PND 30, 45 and 60. Perigonadal fat weight and liver fat content were also evaluated.
Colon epithelial permeability was increased overall in HF rats. By PND 45, the number of colon submucosal ganglia as well as the total number of neurons per area were decreased in HF animals when compared to age-matched controls. By PND 60, HF rats showed significantly higher perigonadal fat weight and fat liver content. The numbers and cell body area of SP, Som, VIP and NPY neurons remained unchanged throughout the experiment.
Further studies should functionally evaluate colon submucosal neurons in HF rats, in order to assess the consequences of such early morphological changes. These data will aid to increase our understanding of the consequences that inadequate nutrition has on adolescent digestive physiology and their potential implications on the ability of enteric submucosal neurons to sense and communicate local information to the brain.

Disclosures: M. JULIO-Pieper: None. **F. Villalobos-Manríquez:** None. **M. Zamorano-Cataldo:** None. **A. López:** None. **N. Cáceres:** None. **J. Eyzaguirre-Velásquez:** None. **J. Escobar-Luna:** None. **J.A. Bravo:** None. **G. Cruz:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.24/S14

Topic: F.07. Autonomic Regulation

Support: FONDECYT Grants #1190729 to J.A.B
FONDECYT Grants #1160398 to R.S-Z
CONICYT "Beca de Doctorado Nacional" Grant # 21180866 to C.G-A
IDRC

Title: Early life gut dysbiosis in rats results in mesocorticolimbic circuit alterations in adulthood

Authors: *C. GONZALEZ-ARANCIBIA[1,2,3], J. ILLANES-GONZALEZ[3], J. URRUTIA-PINONES[3], M. JULIO-PIEPER[2], J. MARTINEZ-PINTO[1], R. SOTOMAYOR-ZARATE[1], J. A. BRAVO[2];
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Abstract: Bacterial colonization of the infant gut begins at birth when partum exposes the newborn to a set of bacteria coming from the mother's gut. Colonization then continues due to environmental factors (i,e.: diet), which goes on to provide microbial richness and diversity. On the other hand, intestinal dysbiosis, defined as a condition in which the microbiome population structure is disturbed, like through the use of medications, resulting in a diminished diversity and richness, is associated with several pathologies, including drug addiction as suggested by recent findings, which correlate changes in microbiota composition to cocaine and methamphetamine use. Preliminary results shows that oral administration of a wide-spectrum non-absorbable antibiotic cocktail (neomycin, bacitracin and vancomycin, all three at a 100mg/Kg dose; and pimaricin, at 5 μg/kg) to a pregnant Sprague-Dawley dam during perinatal period (from embryonic day 18 until postnatal day 7) causes dysbiosis in the male offspring, as evidenced by a decreased intestinal microbial diversity and richness, a condition that remains stable at least until 35 days of age. Interestingly, dysbiosis in the offspring is accompanied by lower dopamine receptor 1 and tyrosine hydroxylase protein levels in nucleus accumbens and ventral tegmental area, respectively. In this work, we aimed to study if early-life exposure to maternal dysbiosis has an effect on dopamine receptor 2 (D2) expression in key areas of the mesocorticolimbic circuit. Furthermore, we evaluated the effect of early-life dysbiosis on male and female offspring at post-natal day 60. There is an increase in glycosylated D2 (D2Glyc) in the prefrontal cortex of males exposed to early-life dysbiosis, an effect that is different from females. However, D2Glyc

and D2 dimer in the nucleus accumbens is reduced in males exposed to early-life dysbiosis in comparison to control. Furthermore, this effect is also observed in the ventral tegmental area. These effects are not observed in females. These results suggests that acquisition of an altered intestinal gut microbiota in early-life affects D2 expression in key areas related to reward behavior, which is persistent until adulthood. Moreover, the effects seem to be more significant on males than females. Therefore, these results suggest that early-life alterations in the development of the microbiota-gut-brain axis might leave the subject more susceptible to develop addictive behaviors later in life.

Disclosures: C. Gonzalez-Arancibia: None. **J. Illanes-Gonzalez:** None. **J. Urrutia-Pinones:** None. **M. Julio-Pieper:** None. **J. Martinez-Pinto:** None. **R. Sotomayor-Zarate:** None. **J.A. Bravo:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.25/S15

Topic: F.07. Autonomic Regulation

Support: NIH Grant R01 DK117508

Title: Somatostatin and npy neurons in the dorsovagal complex of the hindbrain differentially influence gastric motility

Authors: L. BELLUSCI[1], S. VICINI[2], R. A. GILLIS[1], ***N. SAHIBZADA**[3];
[1]Pharmacol. & Physiol., Georgetown Univ., Washington, DC; [2]Dept Parmacology & Physiol., Georgetown Univ. Med. Ctr., Washington, DC; [3]Georgetown Univ. Med. Cent, Washington, DC

Abstract: Somatostatin expressing GABAergic neurons (*Sst-GABA*) are prominently distributed in the dorsalvagal complex, DVC [comprising primarily of the dorsal motor nucleus of the vagus (DMV) and nucleus tractus solitaries (NTS)]. Another neuron present is the neuropeptide Y (Npy) expressing neuron. Our previous study demonstrated that optogenetic stimulation of the *Sst*-GABA neurons *in vitro* results in inhibition of DMV premotor neurons that project to the gastric antrum (Lewin et al, 2016). The **purpose** of the present study was to assess the effects of activation of the *Sst*- and *Npy*- expressing neurons in the DVC on gastric smooth muscle function. To this end, we generated *Sst*- and *Npy*-ChR2 transgenic mice, which were then used to stimulate *Sst* or *Npy* neurons in the DVC. Experiments were performed in urethane-anesthetized mice while monitoring gastric tone and motility. Hindbrains were exposed and *in vivo* light stimulation (pulse train=23; frequency=15 Hz; duration=40ms; power ~1-7 mW; 90s envelope) was performed. Our **results** show that *Sst* and *Npy* neurons affect gastric tone and motility in the

DVC. In ***Sst***: ChR2 mice, optogenetic stimulation of *Sst*-GABA neurons in the DMV decreased motility, a finding that agrees with our earlier *in vitro* results (Lewin, 2016). In contrast, light stimulation of these neurons in the NTS produced an increase in gastric motility. In ***Npy***: ChR2 mice, similar light stimulation of *Npy* neurons in NTS increased the amplitude of gastric motility. In both *Sst* and *Npy* transgenic mice, light-induced responses from the NTS were not abolished by ipsilateral vagotomy indicating that the responses originated from this nucleus and not from the underlying DMV. These results suggest that the activity of both *Sst*-expressing GABA neurons and *Npy*-expressing neurons in the DVC influence gastric function.

Disclosures: L. Bellusci: None. **S. Vicini:** None. **R.A. Gillis:** None. **N. Sahibzada:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.26/S16

Topic: F.07. Autonomic Regulation

Support: NIH Grant R01 DK117508

Title: Npy neurons in the hindbrain regulate vagal neurotransmission to the stomach

Authors: *S. VICINI[1], M. KUAH[2], D. CASTELLANO[2], L. BELLUSCI[3], R. A. GILLIS[3], N. SAHIBZADA[4];
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Abstract: Activity in the hindbrain nuclei that comprise the dorsal vagal complex (DVC) is essential to the regulation of gastric motility. We have previously shown that this activity is greatly influenced by local GABAergic signaling (Herman, et al., 2008) that is primarily due to somatostatin-expressing GABAergic neurons (*Sst*-GABA; Lewin, et al., 2016). To further understand the network dynamics associated with the control of gastric motility in the DVC, we focused on the role of Neuropeptide Y expressing neurons that are prominently distributed in this complex. To this end, we crossed mouse lines that include *Npy*-BAC EGFP, *Npy*-Cre:tdTomato or *Npy*-Cre;ChR2/*Sst*-flip:mCherry transgenic mice to investigate the anatomical and functional characteristics of the *Npy* neurons in the DVC. Our specific objectives were to determine if *Npy* neurons: (1) were part of the gastric vago-vagal circuitry, (2) were inhibitory or excitatory, (3) influence the activity of gastric projecting or other local neurons, and (4) affect end-organ function. To accomplish these, we combined neuroanatomical tracing and optogenetic stimulation with patch clamp electrophysiology to examine the role of these neurons in

transgenic mice of either sex. Our results show that the *Npy* neurons: (1) are part of the gastric vagal circuit as they were trans-synaptically labeled by the viral tracer (PRV-614/RFP) from the gastric antrum; (2) are primarily excitatory as optogenetic stimulation of these neurons evokes EPSCs in gastric-antrum projecting DVC neurons and less reliably in *Sst*-GABA interneurons, which were blocked by NBQX; and, (3) were functionally coupled to each other as evident by an increase in EPSCs on optogenetic stimulation. Furthermore, we observed that *Npy* neurons were reciprocally connected to *Sst*-GABA neurons in the DVC and whose stimulation had a robust inhibitory effect on the action potential firing of the *Npy* neurons. These findings indicate that *Npy* neurons are an integral component of the DVC neuronal network that controls vagal transmission to the stomach.

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Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 071.27/S17

Topic: F.07. Autonomic Regulation

Support: NIH SPARC award 1OT2OD024908
Craig H. Neilsen Foundation, award 476681
NIH award NINDS R01 NS088184

Title: Recruitment of lower urinary tract peripheral afferents and muscles in response to spinal stimulation

Authors: *M. K. JANTZ[1], C. GOPINATH[1], R. KUMAR[1], L. WONG[3], J. I. OGREN[3], G. CHITNIS[3], B. L. MCLAUGHLIN[3], R. A. GAUNT[2];
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Abstract: Bladder dysfunction has a significant impact on daily life and it is therefore important to find effective management strategies. Neurostimulation to modulate bladder reflexes is a potential method to improve function, but the most effective locations to implement a stimulation interface are unclear. Epidural spinal cord stimulation (eSCS) and dorsal root ganglia (DRG) stimulation both have clinical precedent for treating back pain and could target the bladder. Previously, we found that sacral eSCS and DRG stimulation can selectively activate lower urinary tract nerves and preliminary work shows stimulation at both locations can lead to bladder inhibition and contraction. Here we ask whether functionally-relevant EMG responses are

correlated to peripheral afferent activity or function and whether this EMG activity could be used as a surrogate measure of nerve or organ activity.
In anesthetized cats, we placed multichannel epidural arrays (MicroLeads Inc, Ripple LLC) at locations on the sacral spinal cord and cauda equina, or 32-channel microelectrode arrays (Blackrock Microsystems) in the S1, S2 and S3 DRG. To record antidromic compound action potentials resulting from spinal cord stimulation, we placed nerve cuffs on the pelvic nerve and the pudendal nerve and its branches, as well as on the sciatic nerve to measure off-target activation. We placed EMG electrodes to measure motor responses in the external urethral sphincter, anal sphincter, pelvic floor, and gluteal muscle. A bladder and intraurethral catheter measured pressures. We determined threshold stimulation amplitudes at the spinal cord that evoked antidromic activity in the peripheral nerve cuffs and reflexive EMG activity. We also stimulated at predefined amplitudes and frequencies to evoke pressure changes in the bladder and urethra.
We evoked activity in all instrumented nerves and muscles with both eSCS and DRG stimulation. Mean recruitment thresholds were similar for EMG and nerve activity, with animals that tend to have high thresholds for nerve recruitment showing similarly high thresholds for EMG recruitment. Additionally, animals which had a large range between the minimum threshold to recruit a nerve and the amplitude at which all nerves were recruited likewise had a large range between threshold amplitudes for EMG. We compared how antidromic nerve activity compared to the patterns of EMG activity to determine the likely functional effectiveness of stimulation, and further relate this EMG activity to bladder and urethral pressure changes. Ultimately, this could allow the use of EMG as an accessible, rapid clinical metric for effectiveness of stimulation locations and patterns.

Disclosures: **M.K. Jantz:** None. **C. Gopinath:** None. **R. Kumar:** None. **L. Wong:** A. Employment/Salary (full or part-time):; MicroLeads Inc. **J.I. Ogren:** A. Employment/Salary (full or part-time):; MicroLeads Inc. **G. Chitnis:** A. Employment/Salary (full or part-time):; MicroLeads Inc. **B.L. McLaughlin:** A. Employment/Salary (full or part-time):; MicroLeads Inc. **R.A. Gaunt:** None.

Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

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Title: Epidural stimulation and microstimulation of dorsal root ganglion with penetrating microelectrode arrays enables selective access to innervation of lower urinary tract: Mapping and functional outcomes

Authors: *C. GOPINATH[1], M. K. JANTZ[2], R. KUMAR[2], J. I. OGREN[4], G. CHITNIS[4], L. WONG[4], B. MCLAUGHLIN[5], L. E. FISHER[3], R. A. GAUNT[3];
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Abstract: Management of lower urinary tract (LUT) diseases arising out of idiopathic factors and injury remain a challenge in the clinic due to paucity of pharmacological treatment options. Peripheral neuromodulation devices developed to tackle these disorders, such as sacral stimulators, are non-selective with numerous off-target effects, and surgical access may be complex for more targeted peripheral nerve interfaces. Here, we investigate whether epidural stimulation (EPI) and microstimulation of dorsal root ganglion (DRG) through penetrating microarrays, can selectively activate and control reflexes relevant to LUT function. We demonstrate that custom high-density epidural arrays and Utah arrays in the DRG can selectively access LUT innervation, and can modulate bladder, urethral and rectal pressures comparable to peripheral stimulation responses.

To determine the effects of stimulation on peripheral nerve recruitment, isoflurane anesthetized male cats (EPI: n=9, DRG: n=7) were instrumented with nerve cuffs on pelvic nerve, and the pudendal nerve and its branches. Recruitment of the leg was monitored with a sciatic nerve cuff and EMG electrodes in the gluteal muscles. Epidural electrodes were placed under the lumbosacral (L6 and L7 laminae) and sacral (S1 laminae) spinous processes. In separate DRG experiments, 32-channel penetrating Utah arrays were implanted in the S1-S3 DRG. We stimulated on EPI and in the DRG while recording compound antidromic action potentials in nerve cuffs. In a subset of animals under α-chloralose anesthesia (n=2), we monitored the behavioral effects of stimulation using intravesical and transurethral catheters to measure bladder and urethral pressures respectively across various stimulus amplitudes and frequencies.

All the instrumented nerves could be selectively recruited with both stimulation methods, although not every nerve was selectivity recruited in every animal. The stimulation amplitude to evoke functional responses was higher than the minimum threshold to evoke antidromic activity in nerve cuffs. Functional and minimum threshold for EPI was 600-1000 µA and 400-800 µA respectively, while DRG showed 20-45 µA and 2-20 µA respectively. EPI and DRG stimulation evoked bladder contractions similar to direct stimulation of the pelvic nerve. On the same epidural electrodes, at low frequency (3Hz) we observed increased urethral pressure along with suppressed detrusor activity, and at high frequency (33Hz) resulted in high-pressure detrusor-mediated contraction. In the future, we hope to provide a mechanistic understanding of effect of Epidural stimulation and DRG microstimulation on LUT function.

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Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

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Topic: F.07. Autonomic Regulation

Support: NIH SPARC OT2OD025297

Title: A soft silicone electrode net for modulating bladder function

Authors: *R. KUMAR[1], C. GOPINATH[2], T. W. SIMPSON[2], D. M. WEIR[2], M. K. JANTZ[1], A. THIESSEN[3], D. MCDONNALL[3], R. A. GAUNT[2];
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Abstract: Managing lower urinary tract dysfunction is necessary for many people to improve their quality of life. While methods such as peripheral nerve stimulation offer potential to improve function, several challenges, including difficult surgical access to targeted nerves and activation of off-target effects can complicate development. Over the past few decades, bladder wall stimulation has been attempted but challenges with electrode technologies and stimulators have limited performance. To directly regulate the bladder and urethra, we have designed a stretchable electrode array capable of interfacing with an end organ. With a stretchable electrode array that conforms to the bladder wall, we aim to access the hypogastric and pelvic nerve branches on the intramural surface of bladder. This electrode net must accommodate large changes in volume and pressure. Isoflurane-anesthetized cats (n=3) were used for in-vivo testing of conformal strip electrodes, which were used to model an electrode net. Nerve cuffs were placed bilaterally on the hypogastric and pelvic nerves. A transurethral dual-lumen catheter was placed to record pressure and infuse saline into the bladder. The strip electrodes were placed on the dorsal, ventral, and both lateral aspects of the bladder so that the first and last electrode were laid along the base and dome of the bladder, respectively. At an isovolumetric bladder state, each electrode was stimulated while recording bladder pressure and compound action potentials in the instrumented nerves. Electrical impedance was also measured between electrodes on either side of the bladder at various bladder volumes to determine volume-impedance relationships. We also measured the effect of passive net structures on bladder pressure and the relative motion between these nets and the underlying tissue at key locations. Bladder contractions were evoked by stimulation on the surface of the bladder at stimulation amplitudes ranging from 2-8 mA. We found that regions near the uretrovesical junction and bladder dome were most sensitive and that single-electrode stimulation was capable of generating bladder pressures up to 40 cmH2O. Impedance measurements at different frequencies showed a slight increase with increasing volumes on the order of 80 Ohms/5ml at 1 KHz. The relative motion of the net during bladder

volume changes was under 1mm, as measured by the change in distance between markings on the net and nearby tissue landmarks. In future efforts, we hope to design complex mesh patterns based on these inputs and perform functional testing in a chronically implanted animal.

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Poster

071. Autonomic Regulation: Gastrointestinal, Renal, Urinary, and Reproductive Regulation

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Support: NIH SPARC Grant U01 DK116320-01
2018-2019 MnDRIVE Brain Conditions Fellowship

Title: Anatomical and functional mapping of renal nerves

Authors: ***R. TYSHYNSKY**[1], D. VAN HELDEN[2], E. LARSON[1], S. SENSARMA[2], L. VULCHANOVA[1], J. W. OSBORN, Jr[2];
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Abstract: Hypertension is a major health concern throughout the world and is the leading contributor to cardiovascular-related deaths. While drug-based treatments exist, many patients are drug-resistant while others do not adhere to their prescribed treatment. To combat these obstacles, clinical trials are currently underway investigating the efficacy of renal denervation (RDN) for the treatment of hypertension. While recent clinical trials support the concept that RDN is efficacious, it is nonspecifically destructive in nature, ablating both sympathetic (efferent) and sensory (afferent) renal nerves. Furthermore, the specific roles of sympathetic and sensory renal nerves in regulating cardiovascular function is still unclear. As technologies rapidly advance for more targeted ablation of specific types of renal nerves, a thorough mapping of renal nerves, combined with a deeper understanding of the physiological roles of specific nerve fibers, will help guide the development of future therapies using both ablation and neuromodulation technology. With this objective in mind, we are utilizing large volume tissue clearing and imaging techniques, as well as targeted optogenetic modulation of specific renal sympathetic and sensory fiber subtypes. Although it is generally believed that renal afferent nerves primarily innervate the pelvic wall, here we report a close anatomical relationship between renal glomeruli and sensory fibers. To the best of our knowledge, this has not been quantified previously. Approximately half of the glomeruli analyzed are in close proximity to CGRP+ and/or TRPV1+ sensory fibers. These fibers do not appear to penetrate Bowman's

capsules, and they often follow a periglomerular path. Our working hypothesis is that these fibers sense changes in glomerular function, such as glomerular pressure. Ongoing experiments will directly test this hypothesis. Furthermore, experiments investigating the roles of specific renal sensory and sympathetic fibers on cardiovascular and renal function are in progress. Using a custom-designed optogenetic cuff, optogenetic stimulation is applied to periarterial renal nerves in transgenic mice that express channelrhodopsin in subsets of afferent and efferent nerve fibers. To define the functions of these nerve fibers, we are measuring mean arterial pressure and cortical blood flow as physiological outputs of their optogenetic activation. Preliminary results suggest that activation of periarterial TRPV1-expressing sensory fibers in an anesthetized mouse increases mean arterial pressure and ipsilateral renal cortical blood flow.

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Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

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Title: Systemic serotonin-induced inhibition in brown adipose tissue sympathetic nerve activity requires inhibition of the dorsomedial hypothalamus

Authors: *C. M. MOTA[1,2], C. J. MADDEN[1], L. G. S. BRANCO[2], S. F. MORRISON[1]; [1]Dept. of Neurolog. Surgery, Oregon Hlth. & Sci. Univ., Portland, OR; [2]Dept. of Physiol., Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: Systemic administration of serotonergic receptor agonists induces hypothermia. Activation of serotonergic receptors in the central nervous system likely contributes to serotonin (5-hydroxytryptamine, 5-HT)-induced hypothermia, but the specific central thermoregulatory pathways involved in this response are unknown. The dorsomedial hypothalamus (DMH) is an integrative center that plays a key role in supporting brown adipose tissue (BAT) thermogenesis by providing glutamatergic excitation to BAT sympathetic premotor neurons in the rostral raphé pallidus nucleus (RPa). RPa neurons provide glutamatergic and serotonergic inputs to the spinal cholinergic sympathetic preganglionic neurons for BAT which drive stellate ganglion cells that deliver norepinephrine to the interscapular BAT. Thus, activation of this pathway by directly

exciting DMH cells or by skin cooling, increases BAT sympathetic nerve activity (SNA) and BAT thermogenesis. We hypothesized that intravenous (iv) 5-HT reduces thermogenesis by increasing the inhibition of the DMH neurons that are necessary for BAT thermogenic responses. In urethane/α-chloralose-anesthetized, paralyzed, ventilated rats, skin cooling-evoked increases in BAT SNA were inhibited during an infusion of 5-HT (500 µg/h, iv). However, the increase in BAT SNA evoked by injection of N-methyl-D-aspartate (NMDA) (0.2 mM, 60 nl) in the RPa was not blocked by systemic infusion of 5-HT, suggesting that the iv 5-HT-evoked inhibition of BAT SNA occurs at a site antecedent to the BAT sympathetic premotor neurons in the RPa. Blockade of $GABA_A$ receptors by bicuculline (500 µM, 100 nl) in the RPa increases BAT SNA, and this bicuculline-induced increase in BAT SNA was markedly attenuated during an iv infusion of 5-HT. Thus, since the BAT activation response to bicuculline in the RPa is dependent on glutamatergic inputs to the RPa, systemic 5-HT may inhibit a source of glutamatergic input to the RPa. Since the DMH is a source of glutamatergic input to the RPa contributing to skin cooling-evoked BAT SNA and thermogenesis, systemic 5-HT could act to increase the inhibition of thermogenesis-promoting neurons in the DMH. Blockade of $GABA_A$ receptors in the DMH with bicuculline increased BAT SNA and thermogenesis, and this increase was not inhibited by systemic 5-HT. In contrast, the increase in BAT SNA and thermogenesis evoked by glutamatergic (NMDA) activation of DMH neurons was inhibited during iv 5-HT. These data are consistent with the hypothesis that the iv 5-HT-induced inhibition of BAT SNA requires a GABAergic inhibition of BAT sympathoexcitatory neurons in the DMH.

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Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.02/T4

Topic: F.07. Autonomic Regulation

Support: Wellcome Trust (GW4CAT) S133411-105

Title: Torpor trap: Searching for the neural circuit underlying torpor

Authors: *M. T. AMBLER, M. W. JONES, A. E. PICKERING;
Physiology, Pharmacol. & Neurosci., Univ. of Bristol, Bristol, United Kingdom

Abstract: Introduction: Torpor is a hypothermic, hypoactive state, which may be prolonged in hibernators or brief in daily heterotherms. This fascinating state, if mimicked in humans, could have applications as an organ protection strategy in critical illness. This project aims to identify the neural circuits involved in triggering and maintaining torpor in the mouse. **Materials and**

Methods: To identify neurons activated during torpor, 'TRAP-DREADD' mice were generated by breeding TRAP2 (1) mice with RC::L-hM3Dq (2), to generate double-heterozygous offspring. To induce torpor, mice acclimatised to 30°C were subject to 24 hours at an ambient temperature of 18°C, followed by a period of fasting (<16 hours). Torpor, identified using a thermal imaging camera, was defined as a drop in skin temperature of > 2σ below mean fed temperature for > 30 consecutive minutes. For conventional c-fos labelling mice were killed, two hours into a torpor bout, or under control conditions: fasted but not cooled, or cooled but not fasted. TRAP-DREADD mice were given 4-hydroxytamoxifen (4OHT, 5mg/kg i.p) while torpid to induce DREADD expression in active neurons. Torpor in these mice was terminated by feeding 3 hours after injection . After 7 days, mice received clozapine-N-oxide (CNO, 5mg/kg i.p) to reactivate the trapped torpor circuits. **Results:** All female mice undergoing a cooled fast entered torpor (n = 10). Baseline mouse surface temperature at 19°C ambient temperature was 29.7+/-1.4°C, mean nadir surface temperature during torpor was 24.4 +/- 1.4°C. Torpor induced increased c-fos in the posterior hypothalamus, paraventricular thalamus, dorsomedial hypothalamus, and medial preoptic area when compared to both control groups (n=7 per group). The posterior hypothalamus showed statistically significant increase in c-fos expression in torpor compared to both fasting (p = 0.02) and cooling (p = 0.036). TRAP-DREADD mice receiving 4OHT during a torpor bout expressed fluorophore in the same regions (n=3). Injection of CNO induced ‘synthetic’ torpor in the absence of a thermal challenge or fast. **Conclusion:** This work identifies several brain regions that contribute to torpor induction. The TRAP-DREADD approach allows a hypothesis-free search for the neural circuits mediating specific behaviours, and enables reactivation of those circuits in-vivo. Future experiments will use a vectorised approach to target specific regions with a view to finding the minimal circuitry. References: 1. Allen, W. E. et al. Science 357, 1149-1155 (2017). DOI: 10.1126/science.aan6747 2. Sciolino, N. R. et al. Cell Rep. 2016 June 14; 15(11): 2563-2573. DOI: 10.1016/j.celrep.2016.05.034

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Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

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HHMI Investigator Award

Title: Molecular diversity of vagal sensory neurons controlling airway protection

Authors: *B. D. UMANS[1], S. L. PRESCOTT[1], E. K. WILLIAMS[2], N. R. JOSHI[1], S. D. LIBERLES[3];
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Abstract: Homeostasis, or the ability of the body to regulate its internal conditions, is essential for proper cell function and survival. This task is achieved largely by the autonomic nervous system, which regulates bodily functions with exquisite speed and precision. A handful of physiologically-specialized and molecularly-distinct sensory neurons within the vagus nerve comprise a key part of the afferent branch of this system, as they detect both chemical and mechanical cues from many organs in the thoracic and abdominal viscera and convey this information directly to the brain. Vagal signaling is important for a range of vital behaviors including respiration, digestion, cardiovascular control, vocalization, swallowing, and even immune responses; nonetheless, we still know very little about the cellular or molecular diversity of these neurons. Through recent single neuron transcriptome profiling, we have uncovered a myriad of novel, rare vagal sensory subtypes controlling critical, unexplored aspects of mammalian internal homeostasis. In follow-up work, we've focused on new candidate populations that surveil the large airways, and identified a single population with a privileged role in airway protective reflexes. This work may provide important insight to treating those at risk for aspiration pneumonia, choking and dysphagia including the elderly and patients with neurodegenerative diseases.

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Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

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Korea Institute of Oriental Medicine (KIOM) (KSN1812181)

Title: A skin thermal surge following cocaine injection: Mediation of peripheral dopamine receptor

Authors: *S. CHANG[1], Y.-H. RYU[2], S. LEE[1], H. KIM[1], H. JANG[1], D. AHN[1], Y. YI[1], E. JEONG[1], S. YOON[1], C. YANG[1], H. KIM[1];
[1]Daegu Haany Univ., Daegu, Korea, Republic of; [2]Acupuncture, Moxibustion& Meridian Res. Center, Div. of Standard Res., Korea Inst. of Oriental Med., Daejeon, Korea, Republic of

Abstract: Drug addiction has become a worldwide problem, affecting the people of every country in the world. While most studies for drug addiction have been focused on central nervous system including the mesolimbic dopamine (DA) system, peripheral phenomena or the underlying mechanisms are largely unknown. Our preliminary study found that systemic injection of cocaine induced a phenomenon of thermal surge in peripheral skin. Thus, the present study investigated whether the skin thermal surge following cocaine injection is caused by activation of peripheral DA receptor or mesolimbic DA system. The male Sprague-Dawley rats were anesthetized with pentobarbital sodium and peripheral skin temperature was measured using a K-type thermocouple microprobe or an infrared thermal camera. After recording basal temperature for 10 min, animals were given an intraperitoneal injection of cocaine and monitored for up to 30 min after injection. To investigate the mediation of peripheral or central DA receptors, Domperidone (10 mg/kg) or L741,626 (3 mg/kg) was administered 10 min before cocaine injection. Cocaine injection caused a sudden rise of thermal temperature over 3.3 ºC from basal level about 10 min after cocaine injection.
Infrared thermal imaging displayed that the thermal increase was dominant in the distal areas of forelimb and hind limbs, compared to body skin. The temperature increases were blocked by systemic injection of nonspecific or peripheral D2 antagonist. Direct injection of cocaine into brain did not produce thermal changes in skin. These results suggest that a thermal surge in skin following cocaine injection is associated with activation of peripheral D2 receptor, but not central DA receptor.

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Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

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Topic: F.07. Autonomic Regulation

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Title: Vagal afferent nerve activity contributes to the anti-inflammatory effects of vagus nerve stimulation in rats with concanavalin A-induced hepatitis

Authors: *B. JO, K.-W. LEE, C. CHO, H.-R. YOO, *U. NAMGUNG;
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Abstract: Increasing number of studies provide evidence that the vagus nerve stimulation (VNS) dampens inflammation in peripheral organs such as spleen and gastrointestinal tracts, and the principle of cholinergic anti-inflammatory pathway is widely taken as a mechanistic basis on its effect. However, the effects of VNS on the inflammatory liver disease are much less studied. Moreover, vagal connections to liver cells have not been clearly defined at cellular and subcellular levels, and therefore, the contribution of the afferent and efferent components of the vagus nerve to anti-inflammation in liver cells, if any, remains unknown. Here, we prepared a concanavalin A (ConA) model of hepatitis in rats and investigated the regulation on the production of major inflammatory cytokines in the liver after VNS. Acute VNS was administered at the location of the vagal hepatic branch 20 h after intravenous injection of ConA, and brain and liver tissues were analyzed 4 h later. TNF-α, IL-1β, and IL-6 mRNA and proteins were highly induced in the liver of ConA-injected animals and significantly reduced by VNS. To further investigate the effects of VNS on activation of the vagal afferent and efferent nerve fibers, we analyzed choline acetyltransferase (ChAT) in the dorsal motor nucleus of the vagus nerve (DMV). VNS induced robust expression of ChAT protein signals in the DMV neurons that were colocalized mostly with c-Fos. ChAT signals in the DMV were greatly diminished by capsaicin treatment in ConA-treated animals. Capsaicin injection induced caspase 3 signals in neurons of the nodose ganglion (NG), thus suggesting that the removal of NG neurons by capsaicin prohibits VNS-induced ChAT expression in the DMV. Furthermore, VNS-mediated downregulation of TNF-α, IL-1β, and IL-6 production in the liver was significantly weakened by capsaicin administration in ConA-injected animals. Thus, our work provide evidence that the vagal afferent nerve activity is involved in the process of hepatic anti-inflammation which is mediated by VNS in ConA-injected animals.

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Poster

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Support: CIHR

Title: Vasopressin receptor 1a defines mechano and thermosensitive neurons in rat OVLT

Authors: *C. A. ZAELZER, C. W. BOURQUE;
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Abstract: The *organum vasculosum lamina terminalis* (OVLT) is a circumventricular organ that lies in front of the third ventricle where detects variations in systemic osmolality carried by the blood and cerebrospinal fluid (CSF). It contains the product of at least two different mRNAs derived from the TRPV1 gene, *Trpv1* (TRPV1 WT) and *Trpv1dn* (ΔN-TRPV1). The proteins encoded confer different temperature and osmotic sensitivity properties to these neurons (Zaelzer et al. 2015). Recently, using a mix of electrophysiology, single-cell RT-PCR, pharmacology, and temperature stimulation protocols we explored the distribution of the neurons containing those transcripts in an effort to find markers to study differentially the two populations. Our results show a molecularly well-defined population of neurons co-expressing *Trpv1dn* and the Vasopressin Receptor 1a transcript, *Avpr1a*; Patch Clamp analysis shows that these neurons respond to negative pressure, and showed significant reduction in the firing activity after SB366791 was added in the bath. Based on theses findings we engineered a virus with the promoter for *Avpr1a* driving the expression of TdTomato (TOM) and then injected into the OVLT and MnPO areas on rat brains. Fifty days post injection we recorded the responses to negative pressure, temperature, and the immunohistochemistry makeup of the cells positive for TdTomato signal. The results showed a large proportion of TOM neurons display mechano- and thermos sensitivity, immunostaining was used to verify neuronal V1aR (+), TRPV1 (+) specificity of AAV-mediated reporter expression.

Disclosures: C.A. Zaelzer: None. **C.W. Bourque:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.07/T9

Topic: F.07. Autonomic Regulation

Support: The Good Nature Institute
The Florida State University

Title: The effects of ocular atropine administration on c-Fos activation in oxytocin wild-type and knockout mice

Authors: J. GUIDUBALDI, M. A. GREENWOOD, *E. A. HAMMOCK;
Dept of Psychology and Program in Neurosci., Florida State Univ., Tallahassee, FL

Abstract: Dilated pupils are a strong indicator of an aroused nervous system. Heightened arousal is typically thought to cause neural changes that lead to pupil dilation. Recent results from our lab suggest that oxytocin knockout mice display smaller pupils compared to wild type mice, indicative of reduced sympathetic tone. The purpose of this study is to determine if pupil dilation causes neural changes. When administered optically to mice, atropine results in pupil dilation. While the mechanism of atropine at the eye to initiate pupil dilation is well-characterized, the down-stream effects of pupil dilation on brain activity have not been well-characterized. Preliminary data suggests that ocular administration of atropine may also induce some arousal-related behaviors. This study investigated the neural circuit response to atropine administration in the eye. Downstream neural activation was evaluated with c-Fos immunoreactivity. Wild-type C57BL6J male and female adult mice, as well as oxytocin knockout mice were used. We hypothesized the presence of significant neural activation in at least one of two potential sensory pathways down-stream of pupil dilation: the optic nerve and/or the trigeminal nerve. The optic nerve pathway would carry information that results from increased retinal sensitivity after pupil dilation under constant illumination, while the trigeminal pathway would carry information from the mechanosensory stimuli associated with pupil dilation itself. First, we evaluated c-Fos activation in the primary visual cortex. Preliminary results suggest sex by genotype interactions in cell counts contralateral and ipsilateral to the treated eye. Curiously, preliminary results also suggest atropine main effects and a genotype by atropine interaction ipsilateral to the treated eye. Additional circuit-based cell counts are ongoing. From this data, we will be able to elucidate the underlying neural response to pupil dilation, which may allow us to better understand the relationship between pupil dilation and brain activity.

Disclosures: J. Guidubaldi: None. **M.A. Greenwood:** None. **E.A. Hammock:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.08/T10

Topic: F.07. Autonomic Regulation

Support: R01NS099234

Title: A neural projection from the parastrial nucleus to the dorsomedial hypothalamus contributes to the activation of BAT thermogenesis

Authors: *P. CHIAVETTA[1], G. CANO[2], A. STANZANI[3], D. TUPONE[1,3];
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Abstract: The Parastrial nucleus (PS) is a lenticular formation located beneath the anterior commissure, between the bed nucleus of the stria terminalis (BST) and the strial part of the preoptic area. Anatomical and physiological studies have demonstrated 1) a direct projection from PS neurons to DMH, 2) increased cold-evoked Fos expression, and 3) Pseudorabies virus (PRV) infected neurons after injection into the interscapular brown adipose tissue (iBAT). However, it has not been assessed whether a specific population of PS neurons projecting to DMH is specifically involved in the modulation of iBAT thermogenesis. We aim to determine if PS projecting neurons to DMH are selectively responsive to cold or warm stimuli, and if pharmacologic manipulation of these neurons activates iBAT thermogenesis. Two groups of male rats, previously injected with Cholera Toxin subunit-b (CTb) in DMH and FluoroGold (FG) in Raphe Pallidus (RPa), were respectively exposed to warm and cold ambient temperature to elicit Fos expression. A third group of rats, instrumented for recording iBAT sympathetic nerve activity (SNA), skin and core temperature were maintained at 38°C (inhibited thermogenesis) then, pharmacologic manipulation of PS was performed to determine whether PS projecting neurons to DMH modulate iBAT thermogenesis. Immunohistochemical analysis confirmed the existence of direct projections from the PS to DMH and RPa, as well as increased Fos expression in the PS of cold-exposed rats compared to the warm-exposed group. In addition, our data demonstrate that the majority of CTb-ir and a small population of FG-ir neurons in PS express Fos in cold-exposed rats, suggesting the existence of an excitatory input from the PS to DMH and RPa, most likely involved in the control of iBAT thermogenesis. This was consistent with the increased iBAT SNA and temperature observed after bilateral injection of the GABA-A antagonist bicuculline or NMDA in PS. Increased hearth rate and decreased mean arterial pressure were also observed, suggesting a possible involvement of PS in the modulation of cardiovascular activity. Further studies are needed to confirm the role of this nucleus and to determine whether PS neurons are primarily involved in cold evoked thermogenesis or if they are also implicated in stress-induced thermogenesis.

Disclosures: **P. Chiavetta:** None. **G. Cano:** None. **A. Stanzani:** None. **D. Tupone:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.09/T11

Topic: F.07. Autonomic Regulation

Support: NSF ANT-1341602

Title: Behavioral mitigation of detrimental effects of acute thermal stress in Antarctic Nototheinoid fish, N. coriiceps

Authors: *I. I. ISMAILOV[1], J. B. SCHARPING[2], I. E. ANDREEVA[1], M. J. FRIEDLANDER[1];
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Abstract: The Antarctic teleost, *N. coriiceps* lives at temperatures near 0°C, is extremely stenothermic and is thought to be endangered in the face of some of the most rapid environmental changes on earth. Here we report three novel behaviors elicited in these fish by acute thermal stress, presenting as highly reproducible repetitive sequences, in what appears to be a fixed action pattern (FAP). The first behavioral component is composed of continuous cyclical pectoral fin movements, manifesting as a co-mingled “sway” (fins adducted and abducted in counter-phase) or “sweep” (fins adducted and abducted in syn-phase) manner, and may constitute alternative respiratory behavior, facilitating cutaneous and/or branchial gas exchange. The second behavioral component consists of spreading pectoral fins laterally, perpendicular to the trunk, in a splay fashion, maintaining this position for a period of time. Extension of appendages in such manner moves the muscles of pectoral girdle, which may result in expansion of the finite volume of pericardial cavity and increase in cardiac stroke volume. The third behavioral component consists of spontaneous startle-like C-turns, with a short glide. Phases of these C-turns and glides are synchronized with phases of opercular cycles, which may contribute to augmentation of gills irrigation in a manner similar to ram ventilation. In a FAP sequence, splays are followed by short bouts of (1-3 cycles) of alternating “sway”-“sweep” fanning, ending in a C-turn. These reproducible triplet-sequences repeat regularly as often as 5-6 times per minute and represent the majority of motoric behaviors of fish coping with inescapable acute thermal stress. We conclude that these well-orchestrated maneuvers likely emerge from simultaneous optimization of multiple vital physiological functions, complementing each other to possibly contribute to prevention of organismal failure, which ensues when thermal stress is inescapable and extreme.

Disclosures: I.I. Ismailov: None. **J.B. Scharping:** None. **I.E. Andreeva:** None. **M.J. Friedlander:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.10/T12

Topic: F.07. Autonomic Regulation

Support: NIH NS085477
NIH NS072337

Title: EP3R-expressing glutamatergic neurons mediate inflammatory fever

Authors: *N. L. MACHADO[1], S. BANDARU[1], S. B. ABBOTT[2], C. SAPER[1];
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Abstract: Background/Aims: Fever is a critical sign of infectious and inflammatory diseases. During an immune challenge, the neurons in the median preoptic nucleus (MnPO), which express the prostaglandin receptor EP3R ($MnPO^{EP3R}$), are required to produce the stereotypic rise of the body temperature (Tb) - so-called fever. However, the chemical identity of these $MnPO^{EP3R}$ neurons remains unknown. The aim of the present study is to investigate whether GABAergic or Glutamatergic neurons in the MnPO mediate fever responses. Methods and Results: We crossed $EP3R^{flox/flox}$ mice with Vglut2-IRES-cre mice to cause deletion of EP3R from glutamate neurons ($Vglut2/EP3R^{floxed}$); or with Vgat-IRES-cre mice to delete EP3R from GABAergic neurons ($Vgat/EP3R^{floxed}$). These mice and their wild type littermates ($WT/EP3R^{flox}$) received i.p. injections of different doses of LPS or saline as control. LPS caused fever in $Vgat/EP3R^{floxed}$ mice (n=5) that was indistinguishable from their WT littermates (n=4) at low (10µg/kg) (37.15 ± 0.03°C $Vgat/EP3R^{floxed}$ LPS vs. 37.04 ± 0.03°C WT_LPS) and higher dose (100µg/kg) of LPS (37.29 ± 0.05°C $Vgat/EP3R^{floxed}$ LPS vs. 36.91 ± 0.08°C WT_LPS). By contrast, instead of an increase in Tb, LPS produced a hypothermic effect in $Vglut2/EP3R^{floxed}$ mice (n=4) of up to 2.27 ± 0.4°C when compared to saline injection (n=6) or WT (n=3). We then investigated the identity of $MnPO^{EP3R}$ neurons using the RNA-scope in situ hybridization in tissue from $Vglut2/EP3R^{floxed}$, $Vgat/EP3R^{floxed}$ and WT mice. We observed that WT and $Vgat/EP3R^{floxed}$ mice show essentially identical patterns of mRNA for EP3R in the MnPO. However, deletion of EP3R from glutamatergic neurons eliminated the mRNA expression for EP3R in MnPO of $Vglut2/EP3R^{floxed}$ mice. In a separate set of experiments, we then used cell-specific ablation (Cre-dependent AAV-DTA) in the MnPO of Vglut2-IRES-cre ($MnPO^{Vglut2}$) or WT. Ablation of $MnPO^{Vglut2}$ neurons abolished the responses to LPS injection (20µg/kg) in Vglut2-cre mice when compared to saline treatment, while WT mice injected with LPS show the stereotypic fever after LPS injection (Δ°C 0.23 ± 0.02 Vglut2_LPS vs. 0.36 ± 0.04 Vglut2_SAL vs. 1.26 ± 0.05 WT_LPS, n=5 each group). Conclusion: These results demonstrate that $MnPO^{EP3R}$ are exclusively glutamatergic neurons that regulate fever responses.

Disclosures: N.L. Machado: None. **S. Bandaru:** None. **S.B. Abbott:** None. **C. Saper:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.11/T13

Topic: F.07. Autonomic Regulation

Support: ERC Consolidator Grant 2017

SFB 152

Title: The ion channel TRPM2 mediates direct warmth detection within the brain by modulating the sensitivity to temperature of preoptic warm-sensitive neurons

Authors: *G. B. KAMM[1], J. C. BOFFI[3], I. SONNTAG[2], A. TAPPE-THEODOR[1], T. KUNER[2], J. SIEMENS[1];
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Abstract: Body temperature homeostasis relies, among other things, on the activity of specific groups of hypothalamic neurons that receive thermal information from peripherally-located thermoreceptors. Additionally, changes in hypothalamic temperature can trigger appropriated thermoregulatory responses, suggesting that the brain is also endowed with thermoreceptive cells. However, the physiological relevance of central thermosensation in body temperature regulation and the molecular mechanisms underling temperature detection in the hypothalamus are not fully understood. In this work, we introduce a new technique that allows controlled modifications of brain temperature in freely moving mice. Using this novel approach, we generated a spatial map of hypothalamic areas that respond to warmth. We show that mice whose peripheral thermoreceptors have been ablated retain the ability to detect warmth in the preoptic area (POA), a highly temperature-responsive hypothalamic region in mice which plays a central role in thermoregulation. We further observe that brain temperature in the POA mirrors fluctuations in core body temperature such as those associated with circadian cycle or fever. We demonstrate that the ion channel TRPM2 is part of the temperature detection machinery of preoptic warm-sensitive neurons (WSNs), a neuronal population that has been proposed to work as hypothalamic thermoreceptors. Consistent with this hypothesis, TRPM2 knock out mice have a reduced ability to detect warmth in the hypothalamus. Altogether, this study suggests that hypothalamic temperature can reliably and independently from peripheral inputs report body's thermal state. Furthermore, we describe the role of TRPM2 in central warmth detection and provide a causal connection between the activity of preoptic WSNs and central thermosensation.

Disclosures: G.B. Kamm: None. **J.C. Boffi:** None. **I. Sonntag:** None. **A. Tappe-Theodor:** None. **T. Kuner:** None. **J. Siemens:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.12/T14

Topic: F.07. Autonomic Regulation

Support: R01NS069229

Title: Thermoregulation via temperature dependent prostaglandin D2 production in mouse preoptic area

Authors: *T. A. WANG[1], C. TEO[1], M. ÅKERBLOM[3], C. CHEN[1], M. T. FONTAINE[1], V. J. GREINER[3], A. DIAZ[2], M. T. MCMANUS[4], Y. N. JAN[5], L. Y. JAN[6];
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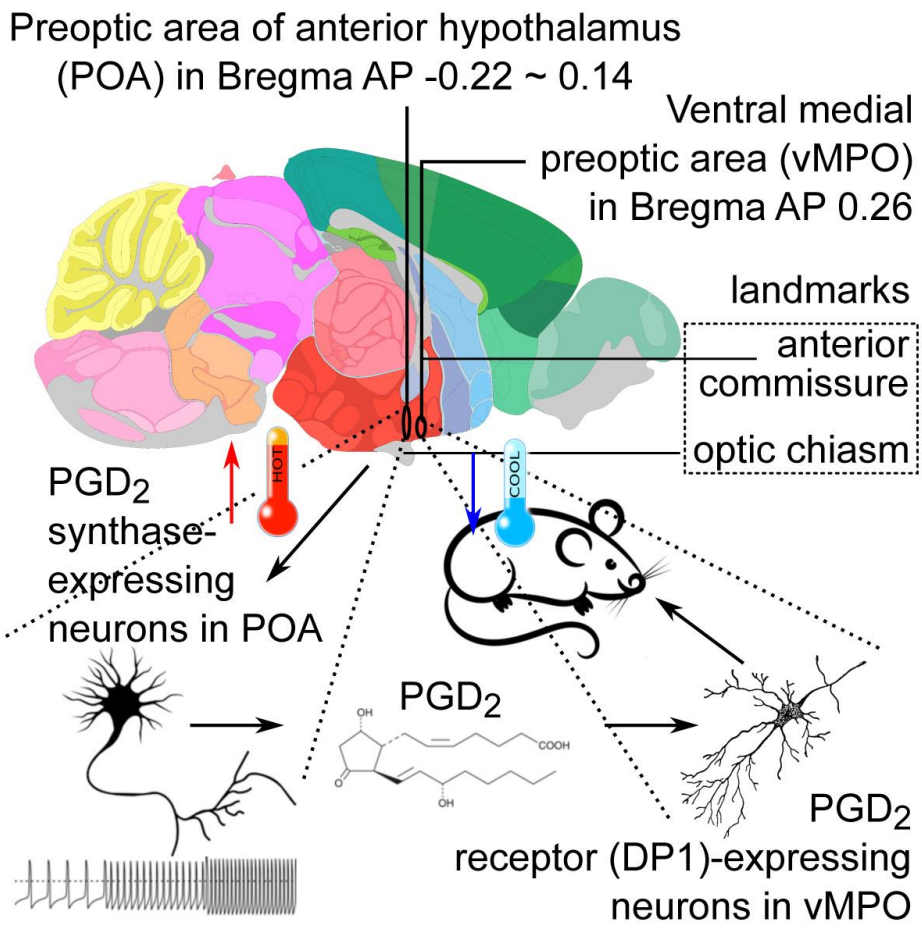
Abstract: Body temperature control is essential for survival. In mammals, thermoregulation is mediated by the preoptic area of anterior hypothalamus (POA), one of the very few brain regions that are thermosensitive. Local temperature change in POA not only alters the spontaneous firing rate of ~30% neurons in this region, namely temperature-sensitive neurons, but also elicits a variety of thermoregulatory responses in live animals. It is still unknown whether and how these temperature-sensitive neurons are involved in thermoregulation, because for decades, they can only be identified via electrophysiological recording. Here we employed the approach of single-cell RNA-seq combined with whole-cell patch-clamp recordings to profile neurons in POA based on their transcriptomes and electrophysiological property. We identified *Ptgds* as a genetic marker for temperature-sensitive neurons. We further showed that activating the *Ptgds*-expressing POA neurons via chemogenetics causes hypothermia in mice, while silencing these neurons leads to hyperthermia. Given that the *Ptgds* gene product, prostaglandin D-synthase (L-PGDS), catalyzes the synthesis of prostaglandin D2 (PGD_2), we tested for PGD_2 involvement in thermoregulation. We found that PGD_2 production in hypothalamic brain slices increases with temperature elevation, while blocking neuronal firing or removing extracellular Ca^{2+} abolishes this temperature dependence. Injection of PGD_2 into the cerebral ventricle of the mouse brain causes hypothermia by activating neurons expressing the PGD_2 receptor DP1 in the ventral medial preoptic area (vMPO). CRISPRi-mediated knock-down of the gene *Ptgds* in POA of the mice leads to disrupted homeostasis of thermoregulation and death of the animals. These findings reveal a negative feedback loop for thermoregulation, with brain temperature elevation promoting PGD_2 production by temperature-sensitive neurons in POA to lower body temperature.



Disclosures: **T.A. Wang:** None. **C. Teo:** None. **M. Åkerblom:** None. **C. Chen:** None. **M.T. Fontaine:** None. **V.J. Greiner:** None. **A. Diaz:** None. **M.T. McManus:** None. **Y.N. Jan:** None. **L.Y. Jan:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.13/T15

Topic: F.07. Autonomic Regulation

Support: NHMRC APP1161029

Title: Neuro-immune cross talk in influenza virus pathogenesis

Authors: K. SHORT[1], B. CHUA[2], C. W. LAW[4], S. B. MAZZONE[3], ***A. E. MCGOVERN**[5]; [1]Sch. of Chem. and Mol. Biosci., Univ. of Queensland, St Lucia, Australia; [2]Dept. of

Microbiology and Immunology, The Peter Doherty Inst. for Infection and Immunity, [3]Anat. and Neurosci., Univ. of Melbourne, Parkville, Australia; [4]Walter and Eliza Hall Inst. of Med. Res., Parkville, Australia; [5]Anat. and Neurosci., The Univ. of Melbourne, Parkville, Australia

Abstract: Influenza virus represents a constant and pervasive threat to human health and the role that pulmonary vagal sensory neurons play in inflammation and viral pathogenesis is yet to be investigated. AIM: To characterise the pro-inflammatory response in pulmonary sensory neurons and determine how they contribute to influenza-induced pulmonary inflammation and disease. RESULTS: Using single cell RNAseq murine pulmonary sensory neurons express receptors and signaling molecules for pro-inflammatory mediators eg. *Ifnar1*/ *2, Tnfrsf1α, Tlr3*. In a murine model (C57Bl/6) of pulmonary infection, Influenza A (PR8 H1N1 strain; 10^5pfu) induced significant transcriptional changes within the vagal sensory ganglia, revealed by RNAseq (114 genes upregulated, n=12) and using single cell qPCR, genes including *Casp1/12, Tnfrsf1α, Ifit1,Ddx58* were significantly upregulated in pulmonary sensory neurons. To examine whether the nervous system could influence IAV infection we performed a unilateral vagotomy disrupting sensory innervation to the right lung lobes. Strikingly, at 4-8 days post-infection vagotomised mice experienced greater weight loss (sham 17.7±1.4%; vagotomy 24.0±1.0%, *p=0.003*, n=10). Vagotomised mice had higher levels of pro-inflammatory cytokines Il-6 (sham 61.3±9.6pg; vagotomy 190.6±23.4pg, *p<0.0001*, n=10) Tnfα (sham 29.6±3.8pg; vagotomy 51.5±8.5pg, *p=0.015*, n=10), MCP-1 (sham 1304.4±137.1pg; vagotomy 2499.2±139.9pg, *p<0.0001*, n=10), Ifnγ (sham 20.0±4.7pg; vagotomy 35.1±6.8pg, *p=0.04*, n=10), and increased viral load (sham 2852.6±843.4 copies/ml; vagotomy 7219.8±1155.2 copies/ml, *p=0.003*, n=10) in their lungs. CONCLUSION: These data provide the first evidence that pulmonary sensory neurons undergo several transcriptional changes and that the autonomic nervous system can modulate the inflammatory response following IAV infection.

Disclosures: K. Short: None. **B. Chua:** None. **C.W. Law:** None. **S.B. Mazzone:** None. **A.E. McGovern:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.14/T16

Topic: F.07. Autonomic Regulation

Support: CNPQ
CAPES
FAPEMIG

Title: Involvement of TRPV1 in thermoregulatory responses in Wistar rats subjected to social stress

Authors: *D. A. CHIANCA, Jr, T. O. REIS, M. T. T. CHIRICO, A. R. R. ABREU, M. R. GUEDES, L. B. T. MESQUITA, P. LIMA, R. C. DE MENEZES;
Federal Univ. of Ouro Preto, Ouro Preto, Brazil

Abstract: The mammals central neural system's response during stress involves increasing blood pressure (BP) and heart rate (HR). It also includes increasing body temperature, termed "stress-induced hyperthermia", which is mediated by a combination of brown adipose tissue (BAT) thermogenesis and caudal artery vasoconstriction. Body temperature is regulated and synchronized by indirect pathways by the TRPV1 channels, which act as a temperature regulation mediator. However the TRPV1 channel in stress-induced hyperthermia has not been determined yet. Thus, we aimed to evaluate the role of peripheral TRPV1 on thermoregulatory and cardiac responses caused social stress. For that, implanted with HR and temperature sensors in all rats, and after 24 hours, they went through a TRPV1 desensitization protocol using resiniferatoxin (RTX). Cardiac and thermoregulatory responses were evaluated after 7 days during an intruder stress paradigm. Our results demonstrated that stress caused tachycardia (+113 ± 11 bpm vs. +118 ± 6 bpm after RTX), body temperature (+1.28 ºC ± 0.32 ºC vs. +1.89 ± 0.35 ºC after RTX) and BAT temperature (+1.35 ± 0.31 ºC vs. +1.67 ± 0.4 ºC after RTX). Importantly, after the stress period HR, body and BAT temperature did not return to baseline levels in the desensitized animals when compared with vehicle (HR: -1 ± 1 bpm vs. +18 ± 3 bpm after RTX, $p<0,05$; BT (+0.3 ºC ± 0.32 ºC vs. +1.82 ± 0.35 ºC after RTX, $p<0,05$; BAT: +0.64 ºC ± 0.16 ºC vs. +1.8 ± 0.3 ºC after RTX, $p<0,05$). Furthermore, we observed that desensitization lowered caudal temperature during stress period contrary to the vehicle group, which increased caudal temperature during stress (+4.4 ± 0.8 ºC after vehicle vs. -1.8 ± 0.2 ºC after RTX) Those results showed that animals desensitized for TRPV1 with RTX do not recover their baseline HR and temperature values after the stress period. Our results suggest that abdominal TRPV1 channels participate in temperature regulation during stress and during recovery after a stress period.

Disclosures: D.A. Chianca: None. **T.O. Reis:** None. **M.T.T. Chirico:** None. **A.R.R. Abreu:** None. **M.R. Guedes:** None. **L.B.T. Mesquita:** None. **P. Lima:** None. **R.C. de Menezes:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.15/T17

Topic: F.07. Autonomic Regulation

Title: Respiratory inflammation increases risk for sudden death in Kcna1-null mice, a model for temporal lobe epilepsy

Authors: *L. NETZEL, J. HALLGREN, K. SIMEONE;
Creighton Univ. Sch. of Medicine, Dept. of Pharmacol., Omaha, NE

Abstract: Rationale: Approximately 1:26 people have epilepsy and around 30% of those cannot effectively treat their seizures with medications, putting them at risk for Sudden Unexpected Death in Epilepsy (SUDEP). Not fully understood, risk factors for SUDEP include generalized tonic-clonic seizures and age. We recently demonstrated the progressive nature of epilepsy and mortality in *Kcna1*$^{-/-}$ (null) mice, a model of temporal lobe epilepsy and SUDEP. We reported respiratory abnormalities in *Kcna1*$^{-/-}$ mice using the MCh challenge, indicating increased breathing rate, apnea and respiratory failure is associated with SUDEP. We determined whether an inflammatory pathology is apparent in lung tissue in younger and older, near-SUDEP ages in *Kcna1*$^{-/-}$ mice and control littermates treated with either control diet or ketogenic diet (KD). The high fat-low carbohydrate ratio KD is the most effective non-surgical treatment for refractory seizures, increasing longevity in the *Kcna1*$^{-/-}$ mouse model, and has been found to restore physiologic abnormalities in *Kcna1*$^{-/-}$ mice. We examined levels of MyD88, NFkB, iNOS, and IFN-γ.
Hypothesis: Increased inflammation in respiratory tissue may associate with respiratory dysfunction in *Kcna1*$^{-/-}$ mice, contributing to SUDEP.
Methods: Immunohistochemistry was performed on alveoli and bronchiole focused regions in the left lobe of 50-µm sections from paraformaldehyde-fixed, 1:1 OTC and 1XPBS inflated mouse lung tissue. Polyclonal antibodies against iNOS, MyD88, NF-kB p65, and IFN-γ were from Abcam. Tissue was incubated with secondary antibody and imaged. Arbitrary fluorescent units were quantified using ImageJ. Differences in florescent density and cell number were analyzed using GraphPad Prism software.
Results: *Kcna1*$^{-/-}$ mice experienced a significant decrease in relative florescent units of iNOS expression in the alveoli ($p < 0.01$). There was no difference in iNOS levels in the bronchiole. No significant difference of MyD88 expression was found in the alveoli or bronchiole-focused regions of the lung. Data from KD groups are in the process of being analyzed.
Conclusion: Data indicates respiratory abnormalities in *Kcna1*$^{-/-}$ mice. Decreased iNOS expression in *Kcna1*$^{-/-}$ tissue may suggest increased TH2 response with increased sensitivity to MCh as *Kcna1*$^{-/-}$ mice approach sudden death. Current studies are examining differences in NF-kB p65 and IFN-γ. Progression of respiratory dysfunction and associated pathology with age may promote respiratory failure when challenged by severe seizures and could influence susceptibility of *Kcna1*$^{-/-}$ mice to sudden death and possibly have implications for refractory epileptic patients.

Disclosures: L. Netzel: None. **J. Hallgren:** None. **K. Simeone:** None.

Poster

072. Autonomic Regulation: Thermoregulation, Inflammation, and Other Interactions

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 072.16/T18

Topic: F.02. Behavioral Neuroendocrinology

Title: Influence of neuraminidase inhibitor on the abnormal jumping off behavior induced experimentally by the combined administration of haloperidol and clonidine in mice

Authors: *N. ONO[1], M. YOSHIDA[2], A. TODA[2];
[1]Miki Hlth. Sci. Res. Inst., Fukuoka, Japan; [2]Hygienic Chem., Daiichi Col. of Pharmaceut. Sci., Fukuoka, Japan

Abstract: Oseltamivir is used clinically in influenza A and B. However, the relationship of this drug to self-injury and delirium in human remains unsolved. In the present study, we clarified whether anti-influenza drugs affect experimentally induced abnormal behavior and further examined the possibility of preventing the behavior. The experimental abnormal behavior evaluated as a phenomenon similar to human, when mice jumped off from high altitude after the treatment with combination of anti-dopamine drug and alpha-2 agonist. Mice were placed onto a circular jumping platform that was 18 cm in diameter and 35 cm in height. When a mouse jumped off, it was immediately returned to the platform. The number of jumps was counted for 40 min after administration of anti-influenza virus drug and the time of first jumping was recorded. Dose of oseltamivir was used in 50, 75 and 100 mg/kg and administered orally 15 min before the combined haloperidol and clonidine treatment. Oseltamivir enhanced the jumping behavior dose-dependently. Each drug used alone at any doses did not present any jumping behavior. Moreover, the enhancement of abnormal jumping off behavior induced by oseltamivir was protected by acetazolamide, phentolamine, diazepam, valproate, fluoxetine or carbamazepine. These effects may be participated in the abnormal response of anti-influenza virus drug with the complicated alternation of catecholaminergic system and the activity of carbonate hydrolyase in the central nervous system at least.

Disclosures: **N. Ono:** None. **M. Yoshida:** None. **A. Toda:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.01/T19

Topic: F.10. Food Intake and Energy Balance

Support: JSPS KAKENHI Grant Number JP19K10039

Title: Analysis of nausea induced by emetine or cisplatin in rats

Authors: *M. FUNAHASHI, Y. HIRAI, K. HISADOME, M. FUJITA, S. SU, N. YAMAZAKI, J. SANEFUJI;
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Abstract: To demonstrate neural mechanisms of nausea induced by emetogenic substances, we investigated emetine- or cisplatin-induced conditioned taste aversion (CTA) in rats receiving vagotomy or lesions of the area postrema. Emetine is the main ingredient of Ipecac that is well-known for its acute emetic effects. Ipecac-induced acute emesis is considered to be induced by gastric mucosal irritation and upregulation of the chemoreceptor trigger zone, however, there is no study elucidate it. Cisplatin is a well-known anticancer drug that shows severe nausea and/or emesis as a marked side effect. The present study investigated a role of vagal nerve activity and neuronal excitability of area postrema neurons in induction of nausea induced by emetine or cisplatin. Male Sprague Dawley rats (250~350 g) were used as experimental animals. We performed experiments in 3 groups of animals: rats received no operation (control), bilateral subdiaphragmatic vagotomy (VX), and lesions of the area postrema (APX). Saccharin was used as a conditioned stimulus, nausea induced by emetine injection (1.0 mM in saline, 10ml/kg, i.p.) or cisplatin (1, 3, 6 mg/kg, desolved in saline, i.p.) were used as an unconditioned stimulus. CTA for saccharin was evaluated using the one bottle method after training period of scheduled drinking, i.e. daily routine consisting of water deprivation for 20 h, voluntary drinking of water or saccharin for 20 min (measurement period), water deprivation for 40 min and voluntary water drinking for 3 h. Emetine-induced CTA was observed in all control rats, and it was abolished in all rats of APX group. In the VX group, although saccharin intake was larger than the control, induction of emetine-induced CTA was statistically significant. Cisplatin (3 mg/kg) induced CTA in all control rats, however, cisplatin (1 mg/kg) failed to induce CTA in some rats. Cisplatin (6 mg/kg) led to bad health such as continuous anorexia for a week and loss of body weight. These data indicate cisplatin (3 mg/kg) is suitable dose for measurement of CTA. These results suggest that emetine-induced nausea may dominantly depend on the neuronal excitability in the area postrema. Such a mechanism of emetine-induced nausea may be different from that of cisplatin-induced nausea, because previous studies have reported a significant role of vagal nerve afferent information in cisplatin-induced nausea and emesis.

Disclosures: **M. Funahashi:** None. **Y. HIrai:** None. **K. Hisadome:** None. **M. Fujita:** None. **S. Su:** None. **N. Yamazaki:** None. **J. Sanefuji:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.02/T20

Topic: F.10. Food Intake and Energy Balance

Title: Neurotrophin-4 is essential for survival of a large proportion of vagal afferents that innervate the small intestinal mucosa

Authors: *H. K. SERLIN[1], E. A. FOX[2];
[2]Dept Psychol Sci., [1]Purdue Univ., West Lafayette, IN

Abstract: Vagal afferents that supply the small intestine play a key role in regulating satiation and gastrointestinal (GI) function. We previously showed that neurotrophin-4 (NT-4) is essential for the survival of vagal mechanoreceptors that innervate the intestinal, but not stomach myenteric ganglia (Fox J Neurosci,2001). Here we use Nav1.8Cre-tdTomato (Nav-Tom) transgenic mice to label vagal afferents (Gautron JCN,2011;Serlin SSIB,2017) and bred them to NT-4 KO (-/-) mice to investigate the effects of NT-4 loss on the vagal afferents innervating the intestinal and stomach mucosa. Immunohistochemical staining of tdTomato was used to label and quantify axons and terminals in the mucosa of the stomach, in villi along the length of the entire small intestine, and adjacent to crypts in the small intestine. These neuronal processes were quantified in NT-4-/-;Nav-Tom, NT-4+/-; Nav-Tom and NT-4+/+; Nav-Tom mice. In the duodenum NT-4-/- mice had a 60% loss of axons entering a villus and 40% loss of terminal branches at villus mid-height compared to NT-4+/- (t = 5.23, t = 4.05, respectively; both $p < .05$) and Nav-Tom (t = 5.83, t = 2.77, respectively; both $p < .05$) mice. There was no difference between NT-4+/- and Nav-Tom mice on either measure (t = .57, $p > .05$, t = .89, $p > .05$). Vagal mucosal stomach afferents, currently being counted, appeared to have a similar innervation density and distribution between all three conditions. These results suggest a large proportion of vagal mucosal afferents innervating the small intestine depend on NT-4 for survival. Loss of vagal afferents to the small intestine muscle and mucosa, involving both mechano- and chemoreceptors, in NT-4-/- mice, supports the organ-specific model of neurotrophin regulation (Brady J Neurosci,1999). It also suggests this mouse model can be used to selectively eliminate vagal afferent innervation of the small intestine without large disruptions to physiology or vagal efferents to study the effects on GI physiology, feeding behavior, microbiota, and diseases in which the vagus is thought to play a role (i.e. Parkinson). We have begun to examine the relationship between the afferent loss in the periphery with the number of vagal terminals in the brainstem, as well as its influences on feeding behavior.

Disclosures: H.K. Serlin: None. **E.A. Fox:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.03/U1

Topic: F.10. Food Intake and Energy Balance

Support: CONACyT PG233918

Title: Peripheral ghrelin elevation and proinflammatory state in dehydration-induced anorexic rats

Authors: *P. SOBERANES-CHAVEZ[1], M. PERELLO[2], E. ALVAREZ-SALAS[1], P. DE GORTARI[1];
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Abstract: Anorexia nervosa (AN) is an eating disorder characterized by decreased food intake despite a dramatic body weight loss and defying increases in peripheral ghrelin levels, the most potent known orexigenic hormone. Besides, anorexic states induced by infectious, neoplastic and autoimmune diseases have been associated to changes in hypothalamic interleukins content; and, rats peripherally or centrally treated with interleukin-1β reduce their food intake, an effect that is reversed by i.c.v. ghrelin injection. In order to define possible mediators of the anorexia in dehydrated rats, we determine plasma ghrelin concentration and arcuate nucleus' interleukin-1β mRNA content in rats with dehydration-induced anorexia (DIA), compared to forced food-restricted (FFR) and to *ad libitum* fed rats. We also assessed morphometric parameters and their correlation with brain and circulating changes. Ninety days old male Wistar rats were divided into three groups (n=10/group): 1) control rats receiving food *ad libitum*; 2) DIA rats, receiving chow *ad libitum* and 2.5% NaCl solution as drinking liquid for 3, 5 or 7 days; 3) FFR rats receiving amounts of food similar to those eaten by DIA rats. As reported, food intake and body weight significantly decreased in DIA and FFR rats since day 1 in comparison to control rats. Abdominal and thoracic circumferences diminished similarly in DIA and FFR rats by day 7 but remained unaffected in days 3 and 5. In contrast, body length did not differ among groups. Body mass index, final body weight, energy intake and food efficiency were lower in DIA and FFR as compared to control rats. As compared to control rats, plasma ghrelin concentration (measured by ELISA) increased similarly between experimental groups. Interleukin-1β mRNA, evaluated in the arcuate nucleus by qPCR, increased in DIA rats as compared to FFR and control rats. This study suggests that a central ghrelin resistance and a proinflammatory state may mediate anorexic behaviour in DIA rats.

Disclosures: P. Soberanes-Chavez: None. **M. Perello:** None. **E. Alvarez-Salas:** None. **P. de Gortari:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.04/U2

Topic: F.10. Food Intake and Energy Balance

Title: Real-time monitoring and quantification of binge-like eating behaviors in animal models

Authors: *Y. FUJIOKA[1], K. KAWAI[1], K. ENDO[1], M. ISHIBASHI[2], S. YOKOI[1], N. IWADE[1], M. KATSUNO[1], H. WATANABE[3], S. ISHIGAKI[1,2,3], G. SOBUE[2];
[1]Neurol., [2]Res. Div. of Dementia and Neurodegenerative Dis., Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; [3]Brain and Mind Reserch Ctr., Nagoya Univ., Nagoya, Japan

Abstract: Changes in dietary behaviors are caused by various stresses or disease conditions. Abnormal eating behaviors have been reported in animal models of certain stress or disease condition. Despite of those clinical etiology, the precise mechanism of eating disorders has not been clarified yet. Most commonly used animal models of binge eating are those established by restriction/refeeding, limited access, and stress schedule models. Using those animals, the quantification of eating behaviors has been usually assessed by the daily consumption of food with modulation of the reward-system by palatable food or opioid. Since obesity and eating disorders are quite common diseases throughout the world, more natural condition and environment would be necessary to assess abnormal eating behaviors underlain the chronic disorders. Herein, we established the real-time monitoring system of eating behaviors using regular diet to quantify the compulsiveness and impaired reward responses in eating. This system revealed that binge-like eating model mice pretreated with intermittent high-fat-diet (HFD) and the mice under single-housing stress showed significantly impaired eating behaviors characterized by deviation in bait selection and elongated bait-approaching duration upon regular chow. These abnormal eating-behaviors were accompanied with aberrant dopamine release at the nucleus accumbens (NAcc) shell before mice showed significant changes in the eating amount. The abnormal eating behaviors of both models were recovered by administration of dopamine to NAcc shell. Thus, the real-time monitoring system we developed can detect abnormal eating behaviors including compulsiveness and reward deficiency of eating caused by impaired mesolimbic dopamine system. The real-time monitoring of eating behavior with regular diet allows us to detect subtle abnormalities that have been previously undetectable, then it can be a biomarker for various neurological and psychiatric disorders and stress conditions.

Disclosures: Y. Fujioka: None. **K. Kawai:** None. **K. Endo:** None. **M. Ishibashi:** None. **S. Yokoi:** None. **N. Iwade:** None. **M. Katsuno:** None. **H. Watanabe:** None. **S. Ishigaki:** None. **G. Sobue:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.05/U3

Topic: F.10. Food Intake and Energy Balance

Support: Startup funds from College of Agricultural and Life Sciences
Startup funds from College of Science

Title: Decreases in DNA methylation in the hypothalamus tracks weight gain during the development of obesity

Authors: *T. MCFADDEN[1], S. A. ORSI[2], J. L. NELSEN[3], M. O'DONNELL[3], T. J. JAROME[4]; [1]Animal and Poultry Sci., [2]Dept. of Biochem., [3]Sch. of Neurosci., [4]Animal and Poultry Sci. and Sch. of Neurosci., Virginia Polytechnic Inst. and State Univ., Blacksburg, VA

Abstract: Within the last thirty years, obesity has reached epidemic proportions, affecting nearly 34% of the U.S. population (Bose 2009). Obesity tends to run in families, suggesting a possible genetic component, and is associated with major health risks such as cancer, heart disease, and diabetes. The hypothalamus is involved in appetite control and altered transcriptional processes in this region can result in the development of obesity, which in turn can lead to further changes in gene expression (Velloso & Schwartz 2012). However, little is known about how the development of obesity leads to lasting changes in hypothalamic transcriptional processes. Epigenetic mechanisms, such as DNA methylation, serves as powerful mechanisms of persistently controlling gene transcription across the lifespan and between generations. Yet it is unknown if epigenetic modifications such as DNA methylation contribute to changes in gene expression during obesity development. Here, we subjected male rats to a high fat diet or standard rat chow diet over the course of six weeks and assessed changes in DNA methylation in the hypothalamus. Rats fed the high fat diet gained significantly more weight than controls, despite lower food intake, and had decreased levels of global DNA 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), a potent transcriptional repressor and activator, respectively, within the hypothalamus. Conversely, DNA 5-mC and 5-hmC levels did not change within another region of the limbic system, the hippocampus, a brain region involved in long-term memory formation, though the high fat animals did display impairments in memory for an object location task relative to controls. Further analysis revealed that in high fat diet animals, but not controls, there was a significant inverse correlation between body weight and DNA 5-mC and 5-hmC levels in the hypothalamus, suggesting that as body weight increased DNA methylation levels decreased. No significant correlations between body weight and DNA methylation levels were observed in the hippocampus of high fat diet or control animals. Collectively, these results suggest that decreases in DNA methylation in the hypothalamus correlate with weight gain,

which might serve as biomarker for the obesity phenotype. Current experiments are testing whether controlling global DNA 5-mC and 5-hmC levels in the hypothalamus using CRISPR-dCas9 can slow or prevent weight gain during the development of obesity. Additionally, we are exploring if female rats have similar hippocampus-dependent memory impairments and decreases in DNA 5-mC and 5-hmC levels within the hypothalamus following weight gain.

Disclosures: T. McFadden: None. **S.A. Orsi:** None. **J.L. Nelsen:** None. **M. O'Donnell:** None. **T.J. Jarome:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.06/U4

Topic: F.10. Food Intake and Energy Balance

Support: The University of California Institute for Mexico and the United States (UC MEXUS)

Title: Levels of the sleep-inducing factor adenosine in blood are modulated in obese patients after blueberry intake

Authors: *E. REYES-CUAPIO[1], M. HIGUERA-HERNÁNDEZ[1], M. PORRUA-ARDURA[1], M. GUTIÉRREZ-MENDOZA[1], H. BUDDE[2], C. BLANCO-CENTURIÓN[3], A. BARCIELA-VERAS[4], N. BARBOSA ROCHA[5], T. YAMAMOTO[6], D. MONTEIRO[7], L. CID[7], D. TELLES-CORREIA[8], S. MACHADO[9], E. MURILLO-RODRÍGUEZ[10];
[1]Univ. Anahuác Mayab, Merida, Mexico; [2]Fac. of Human Sciences, Med. Sch. Hamburg., Hamburg, Germany; [3]Dept. of Psychiatry and Behavioral Sciences.The Med. Univ. of South Carolina., Charleston, South Carolina, SC; [4]Univ. Catolica Dom Bosco, Mato Grosso do Sul, Brazil; [5]Hlth. School, Polytechnic Inst. of Porto, Porto, Portugal; [6]Grad. Sch. of Technology, Industrial and Social Sci. Tokushima Univ., Tokushima, Japan; [7]Sport Sci. Sch. of Rio Maior. Inst. Politécnico de Santarém, Santarém, Portugal; [8]Univ. of Lisbon. Fac. of Med., Lisbon, Portugal; [9]Salgado de Oliveira Univ., Rio de Janeiro, Brazil; [10]Lab. de Neurociencias Moleculares e Integrativas. Escuela de Medicina División Ciencias de la Salud. Univ. Anáhuac Mayab, Mérida, Mexico

Abstract: Obesity is a disease characterized by an excessive accumulation of fat in the body and it has been linked to the enhancement of inflammation-related endogenous molecules, including the sleep-inducing factor adenosine (AD). Since blueberries may induce anti-obesity effects, we tested the hypothesis that blueberries consumption in diet given to obese patients would decrease several obese-related variables, such as weight as well as AD plasma circulating levels. The baseline conditions of obesity-related variables were collected for all subjects prior the

implementation of blueberries consumption. Later, participants received a hypocaloric diet that included the intake of blueberries (50g/day) during 30 days. We found that consumption of blueberries decreased obesity-related variables in men whereas female subjects showed no differences in weight. Importantly, blueberry consumption diminished AD contents in blood in both sexes. Our preliminary data suggest that blueberry intake might decrease AD levels in obese subjects via the action of polyphenols contained in blueberries. However, further studies are needed to support this suggestion.

Disclosures: **E. Reyes-Cuapio:** None. **M. Higuera-Hernández:** None. **M. Porrua-Ardura:** None. **M. Gutiérrez-Mendoza:** None. **H. Budde:** None. **C. Blanco-Centurión:** None. **A. Barciela-Veras:** None. **N. Barbosa Rocha:** None. **T. Yamamoto:** None. **D. Monteiro:** None. **L. Cid:** None. **D. Telles-Correia:** None. **S. Machado:** None. **E. Murillo-Rodríguez:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.07/U5

Topic: F.10. Food Intake and Energy Balance

Support: P50 MH096972
Joel G. Hardman Chair in Pharmacology
Vanderbilt Brain Institute

Title: Modulation of serotonin 2C receptor RNA editing by alterations in energy balance

Authors: *T. MALIK[1], R. B. EMESON[2];
[2]Pharmacol., [1]Vanderbilt Univ., Nashville, TN

Abstract: The 2C-subtype of serotonin receptor ($5HT_{2C}$) has been implicated in a number of human psychiatric and behavioral disorders, including major depressive disorder, dysthymia, obsessive-compulsive disorder, anxiety, and schizophrenia. Despite these numerous roles in behavior, the best characterized function for this receptor involves an anorexigenic response mediated by $5HT_{2C}$ receptors expressed in pro-opiomelanocortin (POMC)-producing cells in the arcuate nucleus of the hypothalamus to maintain energy homeostasis. Transcripts encoding the $5HT_{2C}$ receptor can be differentially modified by adenosine-to-inosine (A-to-I) RNA editing, generating up to 24 protein isoforms that differ in G-protein coupling efficacy and constitutive activity. Widespread disruption of normal $5HT_{2C}$ RNA processing patterns alters feeding and energy homeostasis in mice. Previous attempts to examine dynamic changes in RNA editing profiles from bulk tissue samples have largely proven futile, as many of these studies relied upon population-averaged assays that failed to capture the functional and transcriptional heterogeneity

of unique subpopulations within complex neuronal networks. As the hypothalamus comprises a number of distinct $5HT_{2C}$-expressing cell populations, not all of which are involved in the regulation of energy balance, ensemble measurements have the potential to mask biologically meaningful changes in $5HT_{2C}$ RNA processing occurring in small neuronal subpopulations. Accordingly, we hypothesize that experience-dependent alterations in $5HT_{2C}$ RNA processing occur only within those $5HT_{2C}$-expressing cells directly involved in modulating relevant responses. To investigate whether chronic manipulation of energy balance can dynamically modify $5HT_{2C}$ RNA processing profiles as part of a cellular strategy to refine serotonergic signaling we have used a genetically-engineered mouse model to enrich for $5HT_{2C}$ RNAs expressed selectively in POMC neurons. Here we describe the effect of metabolic perturbations such as high-fat diet, calorie restriction, and exercise on $5HT_{2C}$ RNA editing, specifically in POMC neurons. These studies provide insights into the interplay between $5HT_{2C}$ RNA editing and energy homeostasis.

Disclosures: T. Malik: None. **R.B. Emeson:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.08/U6

Topic: F.10. Food Intake and Energy Balance

Support: National Institute on Alcohol Abuse and Alcoholism under Award Number R00AA021782

Title: Comparison between mouse substrains in a model of binge-like eating

Authors: *G. R. CURTIS, J. R. BARSON;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Binge eating disorder (BED) is the most common eating disorder in the United States and is associated with weight gain and obesity. It is characterized by the consumption of abnormally large quantities of food, particularly food high in sugar or fat, in a short period of time and despite the absence of hunger or compensatory mechanisms. In animal models, binge-like eating is defined by two major criteria: the intake of larger than normal amounts of food and/or the escalation of intake across time. With transgenic mice increasingly used as tools for examining behaviors such as binge eating, it is necessary to design an appropriate mouse model of these behaviors. Previous studies of binge-like eating in mice have used the C57BL/6J substrain; however, many transgenic mice are now bred on a C57BL/6N background. Comparisons between these substrains have revealed that they have differences in metabolism and proneness to obesity, when maintained on a high-fat diet. Thus, it is important to understand

how different mouse substrains behave under models of binge-like eating behavior. The goal of the present experiment was to develop a method in our laboratory to induce binge-like eating in mice and to examine the influence of mouse substrain on this behavior. We gave adult female C57BL/6J and C57BL/6N mice limited access to the highly palatable Chocolate Ensure Plus® for 2 hours/day, 4 days/week (Monday - Thursday) in their home-cage, starting 3.5 hours after the onset of their dark cycle. Subjects had *ad libitum* access to chow and water throughout the experiment. Under this paradigm, both C57BL/6J and C57BL/6N mice reliably demonstrated binge-like eating, escalating their intake across 6 weeks of access. By the end of the experiment, they were consuming nearly 30% of their total daily calories during the 2-hour binging session. Preliminary results suggest that C57BL/6J mice gain more weight than C57BL/6N mice under this paradigm, but the substrains show no difference in Ensure or chow intake. Ongoing experiments are working to validate these findings and to examine chow-consuming control mice. These results indicate that the C57BL/6N mouse is a viable substrain for use in binge-eating experiments, involving limited home-cage access to a highly palatable food.

Disclosures: G.R. Curtis: None. **J.R. Barson:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.09/U7

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant R01DK078749
NIH Grant F30DK117530

Title: The proopiomelanocortin peptide beta-endorphin can mediate the severity of activity-based anorexia in mice

Authors: *C. M. DAIMON, S. T. HENTGES;
Colorado State Univ., Fort Collins, CO

Abstract: Proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus are critical regulators of energy homeostasis such that POMC neuron dysregulation may contribute to the development of disorders of energy balance, including eating disorders. Activity-based anorexia (ABA) is a commonly used rodent model of anorexia (reduced food intake) in which timed, restricted feeding paired with access to a running wheel results in more pronounced reductions in food intake and bodyweight loss, as well as more pronounced increases in wheel running activity compared to animals exposed to either restricted feeding or running alone. While previous studies have suggested a role for POMC neurons in ABA, the specific contribution of POMC neurons to ABA remains unknown. Using fluorescent *in situ*

hybridization, we report here that *Pomc* mRNA increases in mice undergoing ABA compared to free-running, *ad-libitum* fed or sedentary, food-restricted mice. The POMC prohormone is enzymatically cleaved to produce several bioactive peptides, including beta-endorphin. We show that serum levels of beta-endorphin were increased in mice undergoing ABA compared to control animals. Further, increases in beta-endorphin were proportional to the amount of time spent undergoing ABA such that the largest increases in beta-endorphin was observed in animals with the longest exposure to ABA. We next sought to determine the functional consequence of inhibiting the actions of beta-endorphin. Pharmacological antagonism of the primary target of beta-endorphin, the mu opioid receptor, with naloxone produced a bidirectional effect on wheel running activity preceding food presentation (food anticipatory activity, FAA). In mice considered moderate runners during baseline data collection, administration of naloxone during ABA caused a sharp reduction in FAA compared to saline-treated controls. Conversely, administration of naloxone during ABA to mice considered high runners during baseline data collection led to an increase in FAA. Taken together, the results suggest that beta-endorphin is strongly involved in, and can mediate the severity of, ABA in mice.

Disclosures: C.M. Daimon: None. **S.T. Hentges:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.10/U8

Topic: F.10. Food Intake and Energy Balance

Support: CHIR Grant

Title: The growth hormone secretagogue receptor in the ventral tegmental area mediates chronic social defeat induced feeding in mice

Authors: *A. SMITH, L. HYLAND, B. MACAULAY, R. PROWSE, A. ABIZAID; Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: The peptide hormone ghrelin plays a role in regulating feeding behaviors, through its binding to the growth hormone secretagogue receptor (GHSR). In addition to stimulating appetite, ghrelin has also been implicated in regulating stress induced feeding in mice. The ventral tegmental area (VTA) in the mesolimbic dopamine reward pathway displays relatively high expression of the GHSR, and many of these are expressed in dopaminergic neurons. Therefore, it has been hypothesized that ghrelin activation of neurons within the VTA also plays a critical role in regulating feeding behaviors, particularly following exposure to stress. To determine the significance of ghrelin signaling within the VTA in stress induced feeding, we used a pENN.AAV.hSyn.HI.eGFP-Cre.WPRE.SV40 virus to reinstate the expression of the

GHSR in the VTA of GHSR KO mice that only express the GHSR in the presence of cre-recombinase. Control GHSR KO mice received an pAAV.hSyn.eGFP.WPRE.bGH virus. These mice, along with similarly treated WT mice, were then exposed to a 10-day chronic social defeat stress. Results showed that mice with GHSR rescue in the VTA display significantly higher food intake in response to chronic social defeat stress, compared to stressed GHSR KO controls and comparable to WT stressed animals. The results from our preliminary analysis provide strong evidence to suggest that GHSR expression within the VTA plays a vital role in regulating feeding behaviors in response to stress.

Disclosures: A. Smith: None. **L. Hyland:** None. **B. MacAulay:** None. **R. Prowse:** None. **A. Abizaid:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.11/U9

Topic: F.10. Food Intake and Energy Balance

Support: NIDDK R01 DK114008

Title: Hedgehog pathway and ciliary GPCR signaling in obesity

Authors: *S. E. ENGLE, P. J. ANTONELLIS, R. BANSAL, L. S. WHITEHOUSE, N. F. BERBARI;
Dept. of Biol., Indiana Univ. Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Primary cilia are cellular protrusions found on most cell types, including neurons, and their dysfunction results in a range of phenotypes. Bardet-Biedl and Alström syndromes are ciliopathies that present with obesity and type II diabetes. Conditional cilia loss in adult mice also leads to hyperphagia and obesity yet how cilia dysfunction contributes remains controversial and unclear. Primary cilia serve as important signaling centers for the mammalian hedgehog pathway and some G protein coupled-receptors (GPCRs). While the hedgehog pathway is crucial for several aspects of embryonic development, little is known about its role in terminally differentiated neurons. Here we show components of the hedgehog pathway including the ligand sonic hedgehog, the receptor patched, and the mediator smoothened are expressed in the hypothalamus of adult mice. Additionally, we observe detectable levels of pathway ligand in adult serum. Furthermore, expression and serum levels change depending on feeding status. Interestingly, overexpression of a constitutively active form of smoothened *in vivo* in a specific neuronal population results in obesity in mice. These findings suggest a novel role for ciliary hedgehog signaling in the regulation of feeding behavior and adult energy homeostasis. Current studies are focused on assessing the potential for hedgehog agonists and antagonists to influence

neuronal ciliary GPCR signaling, such as signaling through melanin concentrating hormone receptor 1 (Mchr1). Interestingly, we found that pharmacological activation of the hedgehog pathway using the smoothened agonist, SAG, inhibits the ability of hypothalamic primary neurons to respond to melanin concentrating hormone. Understanding how the hedgehog pathway influences ciliary GPCR signaling in terminally differentiated neurons could reveal molecular mechanisms associated with clinical features of ciliopathies, such as hyperphagia-associated obesity.

Disclosures: **S.E. Engle:** None. **P.J. Antonellis:** None. **R. Bansal:** None. **L.S. Whitehouse:** None. **N.F. Berbari:** None.

Poster

073. Feeding and Food-Related Disorders

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 073.12/U10

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant CA207287

Title: Cancer chemotherapeutics targeting Akt-associated pathways evoke emesis in the least shrew

Authors: *N. A. DARMANI, L. BELKACEMI, W. ZHONG;
Basic Med. Sci., Coll Osteo. Med. Pacific, Western Univ. Hlth. Sci., Pomona, CA

Abstract: The PI3K/Akt/mTOR signaling is frequently over-activated in cancers with Akt being pivotal since it influences multiples processes in tumorigenesis. Thus, targeting Akt is an appealing anti-cancer strategy with multiples Akt inhibitors being in clinical development. However, the use of such inhibitors evokes emesis in both patients and vomit-competent animals. The aim of this study is to decipher the role of Akt in emesis using the least shrew (*Cryptotis parva)*. Shrews were injected intraperitoneally with incremental doses of direct (perifosine or pyrvinium) or indirect (rapamycin or temsirolimus) Akt inhibitors and observed for 30 minutes for vomiting behavior. Relative to vehicle-pretreated control (0 mg/kg), perifosine caused significant frequency of vomits (50 mg/kg, $P = 0.0001$) with shrews vomiting by 50% and 90% in response to its 20 ($P < 0.05$) and 50 mg/kg ($P < 0.0005$) doses. The combined administration of non-emetic doses of perifosine (10 mg/kg) and the neurokinin substance P NK_1 receptor selective agonist GR73632 (1 mg/kg) resulted in significantly elevated vomit frequency ($P = 0.01$) and percentage of shews vomiting (75.00%), relative to either perifosine ($P = 0.01$) or GR73632 alone ($P = 0.02$). Pyrvinium also produced dose-dependent increases in vomit frequency ($P = 0.054$) and the percentage of shrews vomiting (50%) at its 5 mg/kg dose ($P = 0.02$). Likewise, rapamycin caused dose-dependent increases in vomit frequency ($P = 0.054$) and

number of shrews vomiting (80%, P = 0.02) at its 10 mg/kg dose, whereas temsirolimus increased the vomit frequency (P = 0.01) and number of shrews vomiting at its 2.5 (57%, P = 0.04), 5 (78%, P = 0.005), and 10 mg/kg (100%, P = 0.001). These findings provide substantial evidence of an emetic role for Akt inhibitors. We postulate that Akt protein inhibition causes over activity of its downstream pro-emetic targets.

Disclosures: **N.A. Darmani:** None. **L. Belkacemi:** None. **W. Zhong:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.01/U11

Topic: G.01. Appetitive and Aversive Learning

Support: ALT1605-2014
ERC-2015-StG 678832
SYNAPSY (51NF40-185897)
SNSF 31003A-176206

Title: A thalamo-amygdalar circuit underlies exposure-induced attenuation of remote fear memories

Authors: ***B. A. SILVA**[1], S. ASTORI[2], A. BURNS[1], H. HEISER[1], M. MARTINEZ-REZA[1], C. SANDI[2], J. GRÄFF[1];
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Abstract: Despite the high prevalence of trauma-related disorders and the consequent need to better understand how long-lasting traumatic memories can be attenuated, the brain circuits supporting this process remain largely unknown. Here, we show - using real-time fiber photometry recordings, bidirectional chemogenetic manipulations and functional circuit mapping in the mouse - that the ventral midline thalamus (VMT) mediates remote fear memory attenuation. During experimental extinction of remote fear memories, we find VMT activity to increase before the end of freezing bouts. Correspondingly, chemogenetically increasing VMT activity ameliorates, while its inhibition impairs remote fear attenuation. Excitatory VMT outputs to the basal amygdala (BA) mediate this mitigating effect, stipulating that the VMT provides instructive safety signals to the BA. These findings identify a critical node for remote fear memory attenuation and foster our understanding of traumatic memory processing.

Disclosures: **B.A. Silva:** None. **S. Astori:** None. **A. Burns:** None. **H. Heiser:** None. **M. Martinez-Reza:** None. **J. Gräff:** None. **C. Sandi:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.02/U12

Topic: G.01. Appetitive and Aversive Learning

Support: RIKEN Brain Science Institute
Howard Hughes Medical Institute
JPB Foundation

Title: Amygdala reward neurons form and store fear extinction memory

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Abstract: The ability to extinguish conditioned fear memory is critical for adaptive control of fear response, and its impairment is a hallmark of emotional disorders like post-traumatic stress disorder (PTSD). Fear extinction is thought to take place when animals form a new memory that suppresses the original fear memory. However, little is known about the nature and the site of the formation and storage of the new extinction memory. Here, we demonstrate that a fear extinction memory engram is formed and stored in a genetically distinct basolateral amygdala (BLA) neuronal population that drive reward behaviors and antagonize the BLA's original fear neurons. The activation of the fear extinction engram neurons and natural reward-responsive neurons overlap extensively in the BLA. Furthermore, these two neuron subsets are mutually interchangeable in driving reward behaviors and fear extinction behaviors. Thus, fear extinction memory is a newly formed reward memory.

Disclosures: X. Zhang: None. **J. Kim:** None. **S. Tonegawa:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.03/U13

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH105851
NIH Grant MH108665

Title: The role of interactions between ventral hippocampus and basolateral amygdala in control of fear extinction memory in mice

Authors: *K. PARK[1], V. Y. BOLSHAKOV[2];
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Abstract: In Pavlovian fear conditioning, fear memory is formed after pairing the conditioned stimulus (CS) with the unconditioned stimulus (US), so that a presentation of the CS after the CS-US association is formed triggers stereotypic physiological responses of escape or avoidance. Fear responses are gradually diminished when the CS is repeatedly presented without the US in a process reflecting new learning termed extinction. Fear extinction is a potential experimental model for exposure therapy, providing a neurobiological background for the development of remedies against fear-related mental disorders. The role of three key brain regions - amygdala, hippocampus, and medial prefrontal cortex - has been highlighted and explored at the molecular, cellular, and synaptic levels in relation to the mechanisms of fear-related behaviors. However, the neural circuit mechanisms of fear control remain to be incompletely understood. Here, we focus on projections from the ventral hippocampus (vHPC) to the basolateral amygdala (BLA) in order to elucidate synaptic and neural circuit mechanisms encoding and mediating fear memory extinction. We found that the vHPC sends monosynaptic glutamatergic excitatory projections to the amygdala, activating both principal neurons and local circuit inhibitory interneurons in the BLA. Fear conditioning resulted in the enhancement of glutamatergic synaptic transmission in the vHPC-BLA pathway, whereas fear extinction was associated with the reduction of excitatory synaptic efficacy but increased feed-forward inhibitory synaptic responses in vHPC-BLA projections. Unexpectedly, we found that activation of vHPC projections also triggers monosynaptic GABAergic inhibitory responses in the amygdala, including the BLA, which are potentiated after extinction of fear memory. Thus, synaptic plasticity mechanisms in vHPC-BLA projections may contribute to the mechanism of fear learning and fear extinction by controlling the balance between excitation and inhibition in the BLA. Taken together, our findings indicate that projections from the vHPC to the BLA may play an essential role in the control of conditioned fear memory.

Disclosures: K. Park: None. **V.Y. Bolshakov:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.04/U14

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant K01MH116158
NSF Grant IOS-1353137
NSF Grant DGE-1313911

Title: Contributions of the postrhinal cortex to retrieval of auditory fear conditioning

Authors: *N. E. DEANGELI, C. A. TOAL, D. J. BUCCI, T. P. TODD;
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Abstract: Although the postrhinal cortex (POR) makes significant contributions to contextual fear conditioning (Burwell, Bucci, Sanborn, & Jutras, 2004), its role in auditory fear conditioning is less clear (Bucci, Phillips, & Burwell, 2000). In two experiments, we examined the role of the postrhinal cortex in the retrieval of auditory fear conditioning; either after conditioning and extinction (Experiment 1) or after conditioning only (Experiment 2).
In Experiment 1, rats received infusions of either inhibitory or control DREADDS into POR. Upon recovery, subjects were exposed to a single 8 min conditioning session in Context A, with three tone-shock pairings. Following a 1-day retention interval, rats were placed back in Context A for 20 min for a context retrieval test. Rats then underwent several days of extinction with 20 presentations of the tone alone in Context B (different visual, olfactory, and tactile cues than Context A) each day. Following the last day of extinction training, rats were re-exposed to Context A for 20 min. On the next two days, fear to the auditory cue was tested via 5 presentations of the CS each day. Each rat received one test session in Context A and one test session in Context B (counterbalanced). Freezing behavior was measured throughout the sessions as an indicator of conditioned fear. POR was inactivated via an IP injection of clozapine-n-oxide (CNO) 30 min before behavior on each renewal test day. Temporary inactivation of POR resulted in reduced freezing to the auditory cue in both Context A and Context B, relative to controls, suggesting that POR is necessary for retrieval of an auditory fear memory following conditioning and extinction.
In Experiment 2, the role of POR was examined following conditioning only. Rats underwent the same treatment as described in Experiment 1 except for the extinction sessions where rats were exposed to Context B but received no tone presentations. Inactivation of POR during the subsequent test sessions resulted in reduced freezing to the tone in both contexts compared to controls. This indicates POR is critical for the retrieval of an auditory fear memory after conditioning. Overall, chemogenetic inactivation of POR impaired retrieval of an auditory fear memory following conditioning (Experiment 2) and conditioning plus extinction (Experiment 1).

Disclosures: **N.E. DeAngeli:** None. **C.A. Toal:** None. **D.J. Bucci:** None. **T.P. Todd:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.05/U15

Topic: G.01. Appetitive and Aversive Learning

Support: DFG - SPP 1665

Title: Subconscious processes during fear extinction in sedated rodents alongside fMRI acquisition are sufficient to reduce long-term fear

Authors: *E. ANDRES[1,2,3], F. AEDO-JURY[1,3], L. HAMZHEPOUR[3], K. RADYUSHKIN[1,4], U. SCHMITT[1,4], R. KALISCH[1,2], A. STROH[1,3];
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Abstract: Adequately responding towards a threat is a crucial mechanism for survival. However, adapting this response when a threat-associated stimulus or situation has become safe requires the formation of an updated (extinction) memory. Upon future encounters with the once threatening stimulus the strength of the extinction memory determines whether or not fear will return. Using Pavlovian fear conditioning, the aim of this work was to develop an extinction model in rats unraveling the brain dynamics during extinction memory consolidation in a sedated, unconscious, state alongside fMRI acquisition and its effect on behavioral adaptation towards the threatening stimulus. One week prior fear conditioning (conditioned stimulus (CS): 30 sec 3 Hz light pulse paired with an electric shock in the last 2 sec) the resting state BOLD signal of medetomidine-sedated Lewis rats was recorded. Five days after fear conditioning, animals were subjected to either extinction (CS presented unpaired in 60 repetitions in the scanner under medetomidine sedation) or sham (resting state only). Using the same stimulus, five days after the second scanning session the freezing rates of both awake experimental groups were measured in a new context. Behaviorally, the freezing rate of rats exposed to visual stimulation in a sedated condition (i.e., the extinction group) was significantly lower in the extinction memory retrieval test compared to the sham group. We demonstrate the feasibility to conduct extinction of fear in the medetomidine-sedated animal within the scanner during fMRI BOLD acquisition, very similar to awake conditions, while brain activity is continuously recorded allowing to investigate whole brain dynamics during this process. These results suggest that besides higher cognitive processes also subconscious neural mechanisms play a major role in the formation of an extinction memory and in adapting the perception and behavior towards the previous threat.

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Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.06/U16

Topic: G.01. Appetitive and Aversive Learning

Title: Extinction reduces fear potentiated startle at long lead intervals but not at short

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Abstract: Conditioned fear is difficult to extinguish, as evident by human fear and anxiety problems. It may be that not all traces of fear are extinguished, even when the extinction procedure *seems* to be working. Some part of the fear response may be hidden in most studies on fear extinction. Hidden, since one usually measures the effect of extinction (and conditioning) in a time window where the response is most pronounced. This time window corresponds to the cognitive, conscious part of the response. The automatic response occurs earlier in relation to the conditioned stimulus, and, hence, is usually not measured. In the present study, an attempt was made to measure both the early, automatic and the later, conscious part of fear conditioning and extinction. As such, fear conditioning, and extinction, assessed by startle modulation, was measured at lead intervals of 200, 1000 and 4000 ms after conditioned stimulus (CS) presentation. Two different tones served as the CS+ and CS-. The CS+ was paired with an electrical finger stimulation on 18 trials, six for each lead interval. In an analysis of block two (i.e. the last three trials of each lead interval), startle was potentiated to all lead intervals following conditioning. However, inhibited startle in the extinction phase was only found at the 1000 and 4000 ms lead interval, but not at the short lead interval (200 ms). These results are supportive of the idea that only parts of the conditioned fear response are extinguished after a *successful* extinction procedure. This offers a possible explanation for the problems with fear extinction, and may be used to improve extinction-based therapies for fear and anxiety disorders.

Disclosures: O. Åsli: None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.07/U17

Topic: G.01. Appetitive and Aversive Learning

Title: Fear learning, slow-wave sleep and trait anxiety: A conditioning study

Authors: *I. C. BIRCH[1], T. B. LONSDORF[3], J. E. DUNSMOOR[4], S. ZAMMIT[2,5], M. W. JONES[6], P. A. LEWIS[1];
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Abstract: Fear conditioning is widely used as a model of fear learning, informing our understanding of post-traumatic stress disorder (PTSD), and other related pathologies. Many of these conditions have been related to sleep disruption, and trait anxiety, however these factors have largely been studied independently. The current study used a fear conditioning, extinction, and reinstatement design to investigate how sleep and anxiety are associated with fear learning. Healthy participants (N = 20, 18 females, aged 19-28) underwent a two-day fear conditioning paradigm. On day 1, participants were successfully conditioned to fear (CS+) and safe (CS-) stimuli, where CS+ trials were paired with an aversive electric shock (56% reinforcement). A night of normal sleep at home was then monitored with wearable EEG. On day 2, participants underwent extinction, reinstatement, and re-extinction. Heart rate variability (HRV) and skin conductance responses (SCRs) were measured during conditioning and extinction phases; SCRs were taken as the main measure of fear learning. Strength of fear conditioning was associated with subsequent sleep, with better learned discrimination between CS+ and CS- associated with a less subsequent slow-wave sleep (SWS), as a proportion of total sleep time, $p = .041$. There was also significant generalisation (loss of fear discrimination) between safe and fear stimuli overnight, which correlated with SWS, $p = .048$. Lastly, trait anxiety predicted increases in SCRs after reinstatement. Specifically, while current anxiety was associated with a greater increase for the CS+, $p = .032$, prospective anxiety was associated with greater increase for the CS-, $p = .015$. HRV was less sensitive to cued conditioning, however a significant discrimination between CS+ and CS- stimuli, which was not present on day 1 learning, emerged after a night of sleep, $p = .047$. Current literature suggests that fear conditioning as a stressful experience impacts subsequent sleep. Here, we show that the strength of fear learning may affect the proportion of subsequent sleep dedicated to SWS, and in turn, this SWS supports consolidation of the distinction between safe and fear stimuli. Further to this, we show that overnight fear consolidation may also be reflected in HRV. In addition, prospective anxiety may predict the generalisation of fear to previously encountered safe items. These data highlight the importance of SWS in fear learning, therefore the findings could support future work investigating sleep manipulation to attenuate fear responses.

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Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.08/U18

Topic: G.01. Appetitive and Aversive Learning

Support: JSPS KAKENHI JP18H01097
JSPS KAKENHI JP16K04419
JSPS KAKENHI JP19H01769

Title: The effects of serotonergic lesion of dorsal raphe nucleus (DRN) and median raphe necleus (MRN) on fear extinction in rats

Authors: *S. NAKAZAWA, T. OZAWA, Y. ICHITANI, K. YAMADA;
Univ. Tsukuba, Tsukuba, Japan

Abstract: In auditory fear conditioning paradigm, animals learn that an auditory cue (conditioned stimulus, CS) predicts the arrival of an aversive stimulus (unconditioned stimulus, US) once they experienced concurrence of CS and US. Importantly, repetitive presentations of only CS reduce CS-evoked fear response. This procedure is called extinction of fear, and is useful to study neural mechanisms of the reduction of fear in animals.
Serotonin is a neuromodulator which has an important role in adaptive neural functions. The midbrain dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) have dense serotonergic neurons, and send a significant amount of projections to the limbic and frontal area such as the hippocampus, amygdala, and prefrontal cortex (Ciranna et al., 2006). Previous studies have shown that serotonin-transporter (SERT) gene knock out (Luoni et al., 2013, Johnson et al., 2019), pharmacological inhibition of SERT (Young et al., 2017, Pedraza et al., 2019), and local or systemic injections of serotonin receptor agonists or antagonists (Catlow et al., 2013, Zhang et al., 2015) could affect fear extinction. However, it is still unclear whether serotonin release fundamentally prevents or facilitates the extinction of fear. Furthermore, the roles of serotonergic neurons in DRN and MRN were never examined. To address this question, in the present study, we tested the effect of serotonergic lesion of DRN and MRN on the fear extinction by injecting the 5,7-dihydroxytryptamine. Based on our results, potential functional dissociation between DRN and MRN serotonergic neurons in fear extinction will be discussed.

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Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.09/U19

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01 MH

Title: The specific effects of CO2 on the lability of fear memory

Authors: *F. NAGHAVI, E. E. KOFFMAN, C. KRUSE, B. LIN, J. DU;
The Univ. of Toledo, Toledo, OH

Abstract: Fear is a survival mechanism occurring in response to life-threatening stimuli. However, inability to control fear is one of the core symptoms of some mental illnesses such as post-traumatic stress disorder (PTSD). Understanding the mechanism of fear can provide us a way to treat these types of illnesses. Pavlovian fear conditioning is a widely accepted method to create associative memory of a conditioned stimulus (CS) and an unconditioned stimulus (US) in lab animals as a model for studies of the biology of fear. Followed by retrieval, an isolated exposure to the CS, the memory returns to a labile state. Therefore, after extinction training, a repeated presentation of the CS in absence of US, during the labile state, that fear-related memory can be destabilized and attenuated. Our previous studies demonstrated that transient acidification via inhalation of 10% CO2 promoted memory lability in mice receiving fear conditioning (tones as CS and foot shocks as US) to more effective extinguish such conditioned fear. To address the specificity of this effect, mice learnt to associate three intermixed tones and three noises with six-foot shocks. After 24 hours, mice received one of the CS or both during retrieval protocol while breathing either 10% CO2 or air, and 30 minutes after retrieval mice underwent extinction. Memory test was performed on these animals 5 days after extinction. Our results showed that after presenting two different CS during fear conditioning, the CO_2 influences only the memory which it has been associated with during retrieval, i.e. CO_2 affect fear memory specifically. Replacing Ca^{2+}-impermeable (CI-) AMPA receptors (AMPARs) with Ca^{2+}-permeable (CP-) AMPARs at the synapse underlies the memory lability associated with retrieval. Electrophysiological analysis of amygdalae from mice receiving above training/treatment showed that when the conditioned memory was retrieved, transient acidification induced a stronger current rectification of AMPARs suggesting an increase in Ca^{2+} permeability mediated by the exchange of AMPARs.

Disclosures: **F. Naghavi:** None. **E.E. Koffman:** None. **C. Kruse:** None. **B. Lin:** None. **J. Du:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.10/U20

Topic: G.01. Appetitive and Aversive Learning

Support: Stony Brook University
Center for Inclusive Education

Title: Subthreshold fear conditioning produces a rapidly developing neural mechanism that primes subsequent learning

Authors: *K. E. COLE[1], J. D. LEE[2], R. G. PARSONS[1];
[1]Stony Brook Univ., Stony Brook, NY; [2]Psychology, Michigan State Univ., East Lansing, MI

Abstract: Prior experiences have the ability to alter the nature of and capacity for future learning. Therefore, identifying the mechanisms that allow prior experience to affect future learning is important in understanding how memories are formed. We have previously shown that a single fear conditioning trial, which on its own does not support long-term memory, primes future learning such that when an identical trial is delivered within 60 minutes or 24 hours, a robust memory is formed. Here, we set out to determine whether or not manipulating neural activity in the amygdala using designer receptors exclusively activated by designer drugs (DREADDs) shortly before or immediately after the initial learning trial would affect the ability of that initial trial to prime future learning. Rats were bilaterally injected with an adeno-associated viral vector expressing a modified form of the human muscarinic receptor M4, hM4Di (AAV8- CaMKII-hM4Di-mCherry) or a control virus (AAV8-CaMKII-eGFP) of the same promoter and serotype, into the BLA. Rats were then fear conditioned with 2 trials spaced by 24 hours. Before the first trial, rats infected with either the inhibitory DREADD receptor hM4Di or control virus were given systemic injections of clozapine-N-oxide (CNO), an inert ligand required to activate the inhibitory DREADD. A third group of rats expressing hM4Di were given injections of the vehicle at the same time point. A second identical trial followed 24 hours later, and memory was tested 48 hours after the final trial. Our results show that inhibiting the amygdala prior to, but not immediately after, the first trial prevented the initial learning from priming future learning. Subsequently, we blocked amygdala activity immediately after the initial training trial by pharmacological inhibition of protein kinase A and mitogen activated protein kinase A; both known to have a more established effect on memory consolidation. Similar to DREADD inhibition, we found that targeting either kinase had no effect on the ability of the initial trial to prime subsequent learning. Together, these results indicate that the neural mechanisms that allow a weak learning event to alter subsequent learning develop rapidly and do not require a post-learning consolidation period.

Disclosures: **K.E. Cole:** None. **J.D. Lee:** None. **R.G. Parsons:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.11/U21

Topic: G.01. Appetitive and Aversive Learning

Support: NIH R56MH 114193
Branch Out Neurological Foundation

Title: Sex differences in the endocannabinoid modulation of fear memory dynamics

Authors: ***A. S. NASTASE**[1], M. MORENA[2], A. SANTORI[3], R. SHANSKY[4], M. N. HILL[1]; [1]Hotchkiss Brain Inst., [2]Cell Biol. & Anat. and Psychiatry, Univ. of Calgary, Calgary, AB, Canada; [3]Physiol. and Pharmacol., Sapienza Univ. of Rome, Rome, Italy; [4]Behavioral Neurosci., Northeastern Univ., Boston, MA

Abstract: Fear extinction is essential for the ability to recover from highly stressful and traumatic events. Impaired fear extinction is believed to contribute to the development and persistence of post-traumatic stress disorder (PTSD). Although women are more likely than men to develop PTSD, there is a paucity of understanding with respect to sex differences in fear extinction. The endocannabinoid (eCB) system - comprised of the cannabinoid type 1 receptor (CB1R), ligands anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), and their respective hydrolyzing enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) - is implicated in the process of fear extinction. We examined whether the eCB system may be involved in modulating fear memory expression and extinction in a sex-specific manner, in rats. We exposed adult Sprague-Dawley rats to an auditory fear conditioning and extinction paradigm and systemically administered the MAGL inhibitor MJN110, the FAAH inhibitor URB597, or vehicle prior to fear extinction in both sexes. Consistent with previous studies we found that males exhibited mostly passive fear responses (freezing), while a large proportion of females engaged in active fear responses (darting). Pharmacological manipulation of eCB signaling did not affect male fear expression. In females, MJN110 decreased freezing while URB597 increased freezing during late fear extinction learning. To determine whether these effects in females were CB1R-mediated, we systemically administered the CB1R antagonist AM251 prior to the enzyme inhibitors. Treatment with AM251 blocked the reduction in freezing induced by MJN110, indicating a CB1R-mediated mechanism. Injection of URB597+AM251, however, further elevated freezing throughout extinction learning as compared to rats treated with URB597 alone. In addition to CB1R, AEA is known to activate transient receptor potential vanilloid type-1 (TRPV1) channels. To determine whether the increase of freezing induced by URB597+AM251 was mediated by activation of TRPV1 receptors, the TRPV1 antagonist

capsazepine (CPZ) was administered systemically together with AM251 and URB597. We found that CPZ rescued the augmentation of freezing induced by URB597+AM251 to levels comparable with controls during extinction learning and recall, suggesting that the URB597 enhancement of freezing was TRPV1-mediated.
These results uncover contrasting roles of the eCB system in mediating fear extinction dynamics between sexes and will help develop sex-specific therapeutic strategies to treat trauma-related disorders that are more common in women than in men.

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Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.12/U22

Topic: G.01. Appetitive and Aversive Learning

Title: Sex differences in prefrontal neural mechanisms underlying fear expression and extinction

Authors: *A. S. RUSSO[1], M. E. VOULO[1], J. LEE[1,2], D. JUN[1], K. KALENJA[1], R. G. PARSONS[1];
[1]Psychology, Stony Brook Univ., Stony Brook, NY; [2]Michigan State Univ., East Lansing, MI

Abstract: Pavlovian fear conditioning occurs when a neutral, conditioned stimulus (CS), such as a light, is paired with an aversive, unconditioned stimulus (UCS), such as a foot-shock. An animal exposed to this paradigm will learn to associate the CS with the UCS such that it will produce a conditioned fear response (CR) to the CS in the absence of the UCS. When multiple presentations of the CS are presented alone, the animal decreases its fear response to the CS, and this is known as extinction. Inability to extinguish a CR is thought to underlie the development of posttraumatic stress disorder (PTSD), and animal studies using fear conditioning have been crucial to identifying the neural mechanisms involved in aberrant fear expression and extinction. Such studies have shown that the prelimbic cortex (PL) is activated during fear recall, and that the infralimbic cortex (IL) is activated during fear extinction. However, most of these studies have used males as subjects, which is problematic because women are almost twice as likely as men to develop PTSD and other fear-based disorders. In order to determine if the function of prefrontal cortical areas in fear recall and extinction is the same for females as it is for males, we exposed adult, male and female Sprague-Dawley rats to a fear-potentiated startle (FPS) paradigm. Rats received fear conditioning (10 x light and foot-shock), a fear recall test (10 x light and startle), extinction training (30 x light alone), and an extinction recall test (10 x light and startle). Fear was measured as an increase in startle when the light was present compared to when the it was absent. One group was sacrificed following fear recall, one following fear

extinction, and a third served as home cage controls. Prefrontal brain slices were exposed to immunohistochemistry with an antibody for mitogen-activated protein kinase (MAPK). In males, we found the expected pattern of results- the extinction recall group showed more MAPK activation in the IL compared to the other groups, and the fear recall group showed more activation in the PL compared to the controls. However, in females, although the fear recall group showed more activation in the PL compared to the other groups, the fear recall group also showed more activation in the IL compared to the other groups, while the extinction recall group did not show elevated activation in the IL. These results suggest that the IL may serve a different function in females than it does in males. Understanding the mechanisms underlying fear in female subjects will be crucial to ensuring that treatments of fear-based disorders, which are often informed by basic knowledge of fear processes, can be effective for both males and females.

Disclosures: **A.S. Russo:** None. **M.E. Voulo:** None. **J. Lee:** None. **D. Jun:** None. **K. Kalenja:** None. **R.G. Parsons:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.13/U23

Topic: G.01. Appetitive and Aversive Learning

Support: NIH 068283

Title: Optogenetic stimulation of substantia nigra to dorsal lateral striatum pathway during fear extinction prevents fear renewal

Authors: *J. WISEMAN, K. SPRAGUE, E. C. LOETZ, A. HOHORST, K. CAMPOS, T. HUBERT, E. B. OLESON, B. N. GREENWOOD;
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Abstract: Exposure therapy relies on the process of fear extinction, which represents new learning that the previous fear-inducing aversive stimulus no longer predicts an aversive response. One limitation of exposure therapy is that fear tends to return in contexts different from the extinction context, a phenomenon called fear renewal. Identification of novel strategies to prevent fear renewal could improve the long-term success of exposure therapy. We have observed that activation of substantia nigra (SN) dopamine (DA) neurons during fear extinction enhances fear extinction recall and blocks fear renewal (Bouchet et al., 2018), but the specific targets in which SN DA acts to enhance fear extinction remain unknown. SN DA neurons projecting to the dorsal lateral striatum (DLS) support the formation of habitual behaviors, which can be resistant to contextual modulation. The goal of the current study was to test the hypothesis

that optogenetic activation of SN terminals in the DLS during fear extinction learning will reduce fear renewal. Adult, male Long-Evans rats received bilateral intra-SN microinjections of control virus or AAV-Chr2-hSyn-mChery and optic ferrule cannulas in the DLS. SN terminals in the DLS were then optogenetically stimulated during auditory fear extinction learning. Fear extinction memory and relapse were subsequently assessed in the absence of stimulation. Results indicate that optogenetic stimulation of DLS-projecting SN neuron terminals during fear extinction reduces the renewal of fear in a novel context while having no effect on extinction memory or spontaneous renewal. Additionally, we verified the effectiveness of the optogenetic stimulation by quantifying cFos expression, a known neural activation marker, in both the DLS and SN with the use of immunohistochemistry. These data suggest that novel therapeutic strategies aimed at SN DLS dopamine circuit could be effective adjuncts to exposure therapy by freeing fear extinction memory from contextual modulation.

Disclosures: J. Wiseman: None. **K. Sprague:** None. **E.C. Loetz:** None. **A. Hohorst:** None. **K. Campos:** None. **T. Hubert:** None. **E.B. Oleson:** None. **B.N. Greenwood:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.14/U24

Topic: G.01. Appetitive and Aversive Learning

Support: 2 T34 GM096958-06
NIH MH114992
Undergraduate Research Opportunities Program

Title: Acute exercise augments fear extinction through a mechanism involving mTOR signaling

Authors: *N. A. MOYA[1], M. K. TANNER[2], A. M. SMITH[1], A. BALOLIA[2], J. K. P. DAVIS[1], E. C. LOETZ[1], B. N. GREENWOOD[1];
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Abstract: Impaired fear extinction, combined with the likelihood of fear relapse following exposure therapy, contributes to the persistence of many trauma-related disorders such as anxiety and post-traumatic stress disorder. Finding mechanisms to enhance the efficacy of fear extinction and to reduce relapse is of utmost importance to mental health. We have observed that a single bout of voluntary exercise either during or immediately after fear extinction learning can enhance the retrieval of fear extinction and reduce relapse. One factor that could contribute to enhanced fear extinction following exercise is the mammalian target of rapamycin (mTOR). mTOR is a translation regulator involved in synaptic plasticity and is sensitive to many exercise signals such as monoamines, growth factors, and metabolic signals. It is also increased after

chronic exercise in brain regions involved in learning and emotional behavior. mTOR is therefore a compelling potential facilitator of the memory-enhancing and overall beneficial effects of exercise on mental health. The goal of the current study was to test the hypothesis that mTOR signaling is critical for the enhancement of fear extinction memory and reduced relapse produced by acute, voluntary exercise. We observed that, like chronic exercise, a single session of voluntary exercise increased mTOR signaling in extinction-related brain areas. Moreover, intracerebral-ventricular (ICV) administration of the mTOR inhibitor rapamycin reduced mTOR signaling and eliminated the enhancement of fear extinction memory produced by acute exercise, without reducing voluntary exercise behavior or altering fear extinction learning. There was no effect on fear renewal. These results suggest that mTOR signaling contributes to acute exercise-augmentation of fear extinction, but more work needs to be done to determine the mechanisms by which exercise reduces fear relapse after extinction. Overall, factors that increase mTOR signaling could be novel targets for the treatment of trauma-related psychiatric disorders.

Disclosures: **N.A. Moya:** None. **M.K. Tanner:** None. **A.M. Smith:** None. **A. Balolia:** None. **J.K.P. Davis:** None. **E.C. Loetz:** None. **B.N. Greenwood:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.15/U25

Topic: G.01. Appetitive and Aversive Learning

Support: NIH 068283
NIH 2 T34 GM096958-06
Undergraduate Research Opportunity Program

Title: Dorsal striatum-modulation of fear extinction and relapse

Authors: *A. BALOLIA[1], M. K. TANNER[1], N. A. MOYA[2], J. DAVIS[2], E. C. LOETZ[2], J. JAIME[2], B. N. GREENWOOD[2];
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Abstract: The poor long-term success of fear extinction-based exposure therapy for post-traumatic stress disorder (PTSD) is often caused by the relapse of previously extinguished fear. We have observed that activation of substantia nigra (SN) dopamine (DA) neurons during fear extinction enhances extinction memory and reduces renewal, one form of relapse during which conditioned fear returns in contexts different from where extinction was learned. Although the specific targets mediating the effects of SN DA on extinction are unknown, their identification could pave the way for the development of novel strategies to reduce fear relapse in PTSD patients. A primary target of SN DA is the dorsal striatum, which consists of two regions: The

dorsomedial striatum (DMS), responsible for goal directed learning, and the dorsolateral striatum (DLS), concerning more inflexible, habitual behaviors. The goal of these experiments was to begin to investigate the roles of the DMS and DLS in fear extinction and renewal in adult, male Long Evans rats. Inactivation of the DLS using a Muscimol/Baclofen cocktail (0.03/0.3 nmol/uL) during auditory fear extinction enhanced fear extinction memory but had no effect on fear renewal, while inactivation of the DMS prevented renewal, but had no effect on extinction memory in the extinction context. These results suggest that the DMS and DLS play different roles in fear extinction and renewal. The DMS supports context-specific fear extinction and, although the contribution of the DLS to normal fear extinction is minimal, fear extinction memory can be rendered resistant to renewal by manipulations that increase the role of the DLS in extinction. Thus, manipulations that increase the involvement of the DLS in fear extinction, such as exercise, could represent effective augmentation strategies for fear extinction in order to reduce relapse of fear in novel contexts. Currently, we are quantifying cFos in the DMS and DLS after inactivation, in order to test the hypothesis that inhibition of one region will increase reliance on the reciprocal region during fear extinction.

Disclosures: **A. Balolia:** None. **M.K. Tanner:** None. **N.A. Moya:** None. **J. Davis:** None. **E.C. Loetz:** None. **J. Jaime:** None. **B.N. Greenwood:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.16/U26

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant GM111725
NIH Grant AG052934

Title: Novelty facilitated extinction ameliorates maladaptive fear learning in the 129S1 mouse strain

Authors: *V. A. CAZARES[1], G. RODRIGUEZ[6], L. J. OUILLETTE[2], R. PARENT[3], K. M. GLANOWSKA[4], S. J. MOORE[5], G. G. MURPHY[1];
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Abstract: Fear conditioning is an associative learning processes by which organisms learn when environmental stimuli are predictive of aversive outcomes. Fear extinction, is the learning process by which organisms learn to reduce defensive responses when the conditioned stimulus no longer predicts the aversive outcome. Fear learning becomes maladaptive when defensive

responses to fear stimuli are overgeneralized to other stimuli or when fear responses persist after extinction training. In humans, maladaptive fear or deficits in extinction learning are core features of generalized anxiety disorder and post-traumatic stress disorder (Fenster et al. 2018; Milad et al. 2009). The 129S1 inbred strain of mice is used as an animal model for maladaptive fear learning because this strain has been shown to generalize fear to other non-aversive stimuli and is less capable of extinguishing fear responses relative to other mouse strains, such as the C57BL/6 (Camp et al., 2009). Here we report that repeated extinction training in novel contexts--a procedure we have termed Novelty Facilitated Extinction (NFE)—ameliorates extinction learning deficits in the 129S1 mouse strain. Moreover, we have leveraged *cfos*-promoter driven YFP expression and tissue-clearing methods to reveal differences in neural activation in the medial prefrontal cortex between innate maladaptive fear exhibit by 129S1 mice, and adaptive fear learning exhibited by C57BL/6 mice. Finally, to gain an understanding of the neural mechanisms underlying the transition between maladaptive to adaptive fear we compare neural activation in the mPFC in 129S1 who have been trained with either standard extinction protocols vs. NFE. Taken together, these studies establish that maladaptive fear in the 129S1 is malleable and can be reduced by NFE training.

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Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.17/U27

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant GM086262
NIH Grant GM111725
NIH Grant AG052934

Title: The role of diminished motivation in extinguishing fear responses to environmental stimuli

Authors: *G. RODRIGUEZ, V. A. CAZARES, T. K. STEVENSON, E. D. GLASS, S. J. MOORE, G. G. MURPHY;
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Abstract: Organisms integrate environmental stimuli with internal states (e.g. motivation, pleasure, fear) to modulate decisions for approach versus defensive behavior. Stressors can affect this decision-making process by decreasing reward-seeking behavior, which can heighten an organism’s chance for survival in certain contexts. Deficits in modulating reward-seeking

behavior, such as an inability to diminish a fearful response when stimuli are no longer predictive of a previously aversive outcome (fear extinction, FE), can be maladaptive and are core characteristics of many anxiety disorders. Previous studies have demonstrated significant variations in the ability for FE learning between wild-type mouse strains (Camp et al., 2009). In particular, the 129S1-inbred mouse strain (129S1) exhibits maladaptive persistent fear (measured as freezing) to a stimulus (a conditioned tone) that no longer predicts an aversive outcome (a footshock). However, 129S1 who have never received a footshock paired to a stimulus also show increased freezing (Cazares et al., 2019). As a result, our hypothesis is that FE deficits in the 129S1 are partially driven by lowered tendencies to perform motivated behavior, which consequently biases their behavior towards defensive responses (vs. approach). To begin to test this hypothesis, we characterized tendencies of 129S1 (relative to C57BL/6, which do not exhibit FE deficits) by monitoring their exploration in home-cage like environments and novel environments, as well as exploration of novel objects. 129S1 exhibit significantly less exploration in both home cage and novel environments. Additionally, when presented with two novel objects, 47% of tested 129S1 exhibit 100% exploration time on one of the two objects. In contrast, no C57BL/6 exhibited 100% exploration time of any of the novel objects. To determine whether the hypo-motivated phenotype in the 129S1 is modulated by the HPA axis response, we are currently testing whether basal and mildly-stressed corticosterone levels differ between strains. Furthermore, we are also testing whether treatment with the selective serotonin reuptake inhibitor, fluoxetine, modulates exploration levels. Due to their apparent innate diminished motivation, we advance the 129S1 mouse strain as an experimental model of anhedonia.

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Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.18/U28

Topic: G.01. Appetitive and Aversive Learning

Support: NSF Grant IOS1456706
NIH Grant MH15947

Title: Examination of diurnal differences in FOS expression in ventromedial prefrontal cortex (vmPFC) to basal medial amygdala (BMA) projection neurons after auditory conditioned fear extinction and conditioned fear recall

Authors: *L. A. MILLISOR, J. R. RAVENEL, M. J. HARTSOCK, H. K. STRNAD, R. RASMUSSEN, R. L. SPENCER;
Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Anxiety disorders, such as PTSD and various phobias share the common symptoms of impaired fear extinction learning and disrupted circadian rhythms. Exposure therapy, which is a form of conditioned fear extinction training is commonly used as a treatment for such disorders. It has been shown that exposure therapy causes better fear extinction when performed during the active phase. The underlying neurological pathways that control conditioned fear and conditioned fear extinction can be studied in rodents using the auditory conditioned fear paradigm. We have previously shown that there is a time of day difference in fear extinction recall. When rats maintained on a 12h:12h light: dark cycle are trained and tested in their active phase, ZT16 (zeitgeber time), they show less fear behavior than rats trained and tested in their inactive phase ZT4. In addition, this diurnal difference in extinction recall requires normal clock gene expression in the vmPFC. (Woodruff et al, eNeuro 0455-18.2018). It has recently been suggested that conditioned fear extinction is under the control of the vmPFC neurons that project to the basomedial amygdala (BMA). It is not known, however, if the time of day difference in extinction recall that we observe is due to a general difference in activation of the vmPFC to BMA neurons over the course of a day. The present ongoing study examines the difference in expression of the early response gene c-Fos in vmPFC to BMA projection neurons between rats trained and tested on an auditory conditioned fear paradigm at ZT16 and ZT4. We find that injection of a retrograde AAV virus with reporter gene into the BMA labels a large population of pyramidal neurons in the ipsilateral infralimbic cortex. For this study both male and female Sprague-Dawley rats are given either auditory fear conditioning or pseudoconditioning. Pseudoconditioning is a control condition, in which rats are exposed to the same auditory fear conditioning procedure but do not receive the foot shock during fear acquisition. For this study, we expect to see more FOS positive vmPFC to BMA neurons in the conditioned rats compared to the pseudoconditioned rats, and more FOS positive vmPFC to BMA neurons in the ZT16 rats compared to the ZT4 rats.

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Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.19/U29

Topic: G.01. Appetitive and Aversive Learning

Support: NSF grant IOS1456706
NIH grant MH15947

Title: Diurnal examination of conditioned fear extinction recall through chemogenetic manipulation of ventromedial prefrontal cortex neurons that project to the basomedial amygdala

Authors: *J. R. RAVENEL, L. A. MILLISOR, C. T. LEVY, S. A. WALLACE, A. B. FAUSNAUGHT, R. A. RASMUSSEN, R. L. SPENCER;
Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Circadian dysregulation and impaired fear extinction are common characteristics of certain neuropsychiatric diseases, such as PTSD. One type of treatment strategy for PTSD is exposure therapy, which is a type of fear extinction training, and has been shown to be more effective at specific times of day in both humans and rodents. Our prior research has shown that recall of conditioned fear extinction training is enhanced in rats housed on a standard 12:12h light:dark cycle that undergo extinction training and recall testing during their active phase at ZT16 (ZT=zeitgeber time) compared to rats trained and tested during their inactive phase at ZT4. This enhanced extinction recall at ZT16 is dependent on an intact molecular clock in the ventromedial prefrontal cortex (vmPFC) since knockdown of Per1/Per2 with a targeted AAV-shRNA eliminates the enhancement (Woodruff et al., eNeuro 0455-18.2018). Recent studies suggest that there is positive regulation of extinction processes by the vmPFC, specifically neurons that project to the basomedial amygdala (BMA). It is unknown whether the enhancement of extinction fear recall at ZT16 is caused by an increase in overall activity of BMA projecting vmPFC neurons during training or testing at that time of day. To understand how modulation of this pathway can affect fear extinction learning, we utilized a chemogenetic approach in Sprague Dawley rats to activate or silence these BMA projecting vmPFC neurons. An intersectional viral strategy was employed in which a retrogradely transported virus encoding cre recombinase (AAVrg-pmSyn1-EBFP-Cre) was injected into the BMA and a cre-dependent virus encoding a DREADD receptor (AAV2/8-hSyn-DIO-hM3Dq-mCherry or AAV8-hSyn-DIO-hM4Di-mCherry) was injected into the vmPFC. Using this approach, we showed that silencing of this pathway at ZT16 with a Gi-coupled DREADD during extinction training resulted in decreased freezing to the cue. We are currently testing the effects of activation of this pathway in both male and female rats with a Gq-coupled DREADD during either extinction training or extinction recall testing. This study will determine if modulation of the overall activity of this pathway is responsible for the enhancement in extinction recall observed at ZT16.

Disclosures: J.R. Ravenel: None. **L.A. Millisor:** None. **C.T. Levy:** None. **S.A. Wallace:** None. **A.B. Fausnaught:** None. **R.A. Rasmussen:** None. **R.L. Spencer:** None.

Poster

074. Fear and Aversive Learning and Memory: Extinction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 074.20/U30

Topic: G.01. Appetitive and Aversive Learning

Support: NSF Grant IOS1456706

NIH Grant MH15947
NSF GRFP Grant DGE144083

Title: Diurnal examination of infralimbic prefrontal cortex neuronal activity: Role of projections to the basomedial amygdala during auditory conditioned fear extinction in rats

Authors: *M. J. HARTSOCK, M. J. NAVARRO, N. A. BRENNAN, J. R. RAVENEL, L. A. MILLISOR, H. K. STRNAD, M. P. SADDORIS, R. L. SPENCER;
Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Stress-related mental disorders are associated with circadian disruptions and with impairments in fear extinction learning. In rats, projections from the infralimbic prefrontal cortex (IL) to the basomedial amygdala (BMA) are believed to mediate auditory fear extinction. We have shown recently that rats exhibit a diurnal rhythm in fear extinction recall that is abolished by disruption of the IL circadian system (Woodruff et al, eNeuro 0455-18.2018). To determine whether diurnal rhythms in extinction may be associated with diurnal rhythms of neural activity in the IL, we recorded IL neural activity during auditory conditioned fear extinction at zeitgeber time (ZT) 4 (inactive phase) or ZT16 (active phase). Adult male Sprague-Dawley rats on a 12h:12h light:dark cycle received two bilateral microinfusions: a retrogradely-transported cre recombinase (AAVrg-pmSyn1-EBFP-Cre) into the BMA and a cre-dependent channelrhodopsin (AAV5-EF1α-DIO-ChR2-EYFP) into the IL to enable selective identification of IL-to-BMA pyramidal neurons. Three months later, 8-channel electrode arrays surrounding an optical fiber were implanted in the IL in each hemisphere. After recovering from surgery for two weeks, rats were trained and tested in a delayed auditory fear conditioning protocol consisting of tone-shock pairing on Day 1, fear extinction on Day 2, and fear extinction recall on Day 3. *In vivo* electrophysiological recordings in IL were conducted on Days 2 and 3. Additionally, we optically stimulated IL-to-BMA neurons (470nm, 20Hz, 5ms pulse, 5mW) during extinction recall on Day 3. Although rats achieved equal fear conditioning at ZT4 and ZT16, freezing behavior was significantly greater at ZT4 on Days 2 and 3, and optical stimulation had limited effect. We identified phasic neural activity associated with both cue presentation and behavioral responding (i.e. freezing or unfreezing). For behavioral responses, we typically saw oppositely-valenced phasic neural responses for freezing onset and offset that anticipated the behavioral action by 500-1000 milliseconds. This pattern was also seen in local field potentials taken in the same location. Using optogenetic stimulation with simultaneous electrophysiological recording, we have identified recorded neurons that are positive for ChR2, indicating projections from the IL to the BMA. Ongoing assessments will evaluate time-of-day differences in neural activity and determine the involvement of IL-to-BMA neurons in the encoding of cues and behavioral events.

Disclosures: M.J. Hartsock: None. **M.J. Navarro:** None. **N.A. Brennan:** None. **J.R. Ravenel:** None. **L.A. Millisor:** None. **H.K. Strnad:** None. **M.P. Saddoris:** None. **R.L. Spencer:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.01/U31

Topic: G.02. Motivation

Support: NIH grant: R00 AA021417
NIH grant: 1F31DA047050-01
NIH grant: T32 NS 63391-13
NIH grant: R25 GM 55036-19

Title: Excitatory input from the insula to the ventral bed nucleus of the stria terminalis governs the acquisition of cues that predict reward

Authors: *K. S. GIRVEN[1], S. ARONI[2], P. N. MCKEON[1], J. F. CHEER[3], D. R. SPARTA[4]; [1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Dept. of Anat. and Neurobio., Univ. of Maryland, Sch. of Med., Baltimore, MD; [3]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [4]Univ. of Maryland, Baltimore, MD

Abstract: Individuals suffering from substance use disorder often experience relapse events that are attributed to drug craving. Insular cortex (IC) function has been implicated in processing drug-predictive cues and is thought to be a critical substrate for drug craving. Here, we uncover the functional connectivity of a novel projection from the IC to the ventral bed nucleus of the stria terminalis (vBNST), a portion of the extended amygdala that modulates dopaminergic activity within the ventral tegmental area, and investigate the role of this pathway in establishing reward-predictive cues. We reasoned that these cues activate IC projections that synapse onto projection neurons within the vBNST, which then activate the mesolimbic dopamine pathway resulting in the acquisition of associations between exteroceptive stimuli and rewards. To examine this hypothesis we utilized in vivo optogenetics to bidirectionally control the IC-vBNST projection in various behavioral paradigms. We found that the IC is connected to the vBNST via excitatory projections, that when activated, result in a real-time place preference (RTPP) and sustain intracranial self-stimulation (ICSS). We also determined that dopamine neurotransmission is required for these effects. We then silenced the IC-vBNST projection and found that it is necessary for the acquisition of cue-reward pairs, but not for the maintenance of established reward-predictive cues. Thus, the IC-vBNST projection is sufficient to drive reward-related behavior and necessary for establishing reward-predictive cues, making this pathway a previously undescribed target for addictive substances to usurp the brain's natural reward systems.

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Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.02/U32

Topic: G.02. Motivation

Support: DFG HA2340/11-1

Title: Effects of disconnection of the medial orbitofrontal cortex and ventral tegmental area on effort-related responding in rats

Authors: *W. HAUBER, A. MUENSTER;
Univ. Stuttgart/Dept Neurobiol, Stuttgart, Germany

Abstract: Nucleus accumbens (NAC), ventral tegmental area (VTA) and mesoaccumbens dopamine (DA) neurons are major components of the neural circuitry subserving effort-related motivational function. Recent evidence suggests that the medial orbitofrontal cortex (mOFC) may be another brain area that mediates the ability of an organism to work with vigor towards a selected goal. For instance, in rats, pharmacological inhibition of the mOFC increased, while pharmacological stimulation of the mOFC reduced responding under a progressive ratio (PR) schedule of reinforcement[1]. Here we analyzed in more detail the role of the mOFC and interactions between mOFC and VTA and NAC in PR responding. In Experiment 1, we used an optogenetics approach to globally stimulate mOFC neurons prior to testing rats on a PR task. Results demonstrate that, relative to controls, pre-test optogenetic mOFC stimulation reduced PR responding. This finding is consistent with previous data showing that pharmacological mOFC stimulation reduced PR responding[1]. In Experiment 2, we used a pharmacological disconnection approach to analyze the role of neural pathways linking the mOFC and VTA in PR responding. Specifically, we tested the effect of a unilateral pharmacological inhibition of the mOFC in combination with a contralateral pharmacological inhibition of the VTA. Results indicate that rats subjected to a disconnection between the mOFC and VTA display increased PR responding. In Experiment 3, we used a combined pharmacological/neurotoxin inactivation disconnection approach to analyze the role neural pathways linking the mOFC and VTA dopamine neurons in PR responding. Results demonstrate that rats subjected to a disconnection between the mOFC and VTA dopamine neurons show increased PR responding. Collectively, Experiment 2 and 3 suggest that the information transfer between mOFC and VTA DA neurons is not essential for effort-related responding. Taken together, our data provide further support to the notion that the mOFC plays a key role in effort-related responding. Furthermore, our findings implicate that the mOFC might control effort-related responding not necessarily by interacting with mesoaccumbens DA neurons. The idea that the mOFC is a key part of the neural circuitry that governs effort-related responding in rats is in line functional imaging data in humans[2] and may

provide further understanding of the neural basis of effort-related dysfunction observed in several psychiatric disorders[3]. [1] Münster A, Hauber W. *Cereb Cortex* 2018; 28:4379-4389 [2] Park IH, Lee BC, Kim J-J, Kim J Il, Koo M-S. *J Neurosci*. 2017;37:4370-4380 [3] Salamone JD, Correa M. *Neuron* 2012;76:470-485

Disclosures: W. Hauber: None. **A. Muenster:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

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Program #/Poster #: 075.03/U33

Topic: G.02. Motivation

Support: NIH DA040280
Charles E. Kubly Mental Health Research Center

Title: Pavlovian conditioned approach behavior is encoded by cortico-accumbens activity

Authors: *M. G. SPRING, J. R. MCREYNOLDS, A. J. CACCAMISE, K. SONI, E. A. PANTHER, B. M. WINDSOR, J. R. MANTSCH, R. A. WHEELER;
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Abstract: Cortical inputs into the nucleus accumbens (NAc) subserve an organism's ability to engage in goal directed action. This pathway has been well studied in instrumental forms of learning, but less is known about its involvement in the approach of classically conditioned cues. To study this latter form of appetitive learning, *in vivo* single unit electrophysiology and fiber photometry were used to monitor prelimbic PFC (PL) or PL-NAc activity during a Pavlovian conditioned autoshaping task. In the autoshaping task the presentation of a CS+ or CS- lever predicted the delivery of a sucrose pellet or no reward delivery, respectively. Animals in this task acquire either a learned interaction with the cue itself or a conditioned approach of the pellet delivery cup in response to the presence of the CS+ lever. In the electrophysiology experiment, adult male Sprague Dawley rats were trained for 10 days in the autoshaping task. Those that developed CS+ approach (n=9) were implanted with steel microwire arrays targeting the PL. Following surgical recovery, single unit activity was recorded during a single autoshaping session. The majority of PL units responded to the CS+ (45/70) and a majority of these did so without responding to the CS- (31/45). In addition, while the predominant response of these units was a reduction in firing rate (24/31), units displaying elevated firing rates were more frequently observed in animals displaying greater CS+ than goal approach. This finding was particularly interesting in light of recent research indicating that PL efferents targeting the NAc are predominantly excited by reward conditioned stimuli and promote goal-directed behavior. Therefore, an intersectional photometric approach was used to selectively monitor GCaMP

fluorescence in cells of this PL-NAc pathway during autoshaping acquisition. In the photometry experiment, retrograde AAV containing Cre-recombinase was infused in the NAc core and Cre-dependent GcAMP6f was infused into the PL of adult male Sprague Dawley rats (n=8). An optic fiber was implanted into the PL to allow for the characterization of pathway-specific PL-NAc activity. During autoshaping acquisition, CS+ presentation consistently evoked a robust increase in calcium signal that emerged across training sessions, demonstrating the encoding of cues that promote conditioned approach by NAc-projecting PL neurons. Ongoing studies are aimed at characterizing the nature of this signal and its role in regulating CS+ approach behavior.

Disclosures: **M.G. Spring:** None. **J.R. McReynolds:** None. **A.J. Caccamise:** None. **K. Soni:** None. **E.A. Panther:** None. **B.M. Windsor:** None. **J.R. Mantsch:** None. **R.A. Wheeler:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.04/U34

Topic: G.02. Motivation

Support: NIH NIDA-IRP

Title: The medial prefrontal cortex and anteromedial thalamic nucleus organize a positive feedback loop and regulate motivation of anticipatory behavior

Authors: S. JUNN, L. R. WHITAKER, C. NICOLAS, ***S. IKEMOTO**;
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Abstract: The prefrontal cortex (PFC) plays an executive role in the production of anticipatory (also referred to as voluntary or goal-directed) behavior. Such function is thought to arise from the activity of the PFC projecting to the basal ganglia via the dorsal and ventral striatum. In addition to such projections, Alexander and Crutcher's classic circuit model (1990) outlines PFC's direct projections to the thalamus in anticipatory behavior. However, little is known about the contribution of the thalamic nuclei in anticipatory behavior. Using optogenetics, brain slice electrophysiology and intracranial self-stimulation, we investigated the role of the thalamus in motivational control of anticipatory behavior triggered by medial PFC (mPFC) stimulation in mice. We found that mice learned to press a lever that delivered photostimulation exciting the terminals of mPFC neurons within the anteromedial thalamic nucleus (mPFC-to-AM neurons). Mice also learned to deliver photostimulation exciting AM neurons or exciting the terminals of AM neurons in the mPFC (AM-to-mPFC neurons). These results suggest that the activation of mPFC-to-AM or AM-to-mPFC neurons elicits reward that triggers anticipatory behavior. Brain-slice electrophysiology revealed that photostimulation of mPFC-to-AM neurons excited AM-to-mPFC neurons and that photostimulation of AM-to-mPFC neurons excited mPFC-to-AM

neurons, suggesting that mPFC and AM neurons organize a positive feedback loop. Together, these results suggest that AM neurons are reciprocally connected with mPFC neurons, forming a functional loop for regulating motivation of anticipatory behavior. Thus, mPFC neurons appear to regulate motivation of anticipatory behavior through not only their projections to the basal ganglia, but also those to the thalamus, particularly the AM, organizing a positive feedback loop.

Disclosures: S. Junn: None. **L.R. Whitaker:** None. **C. Nicolas:** None. **S. Ikemoto:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.05/U35

Topic: G.02. Motivation

Support: NIH NIDA-IRP

Title: Medial prefrontal cortex stimulation produces reward and activate dopamine neurons via nucleus accumbens neurons projecting to the ventral tegmental area and substantia nigra

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Abstract: The medial prefrontal cortex (mPFC) regulates motivation of anticipatory behavior. Such function is thought to arise from the activity of the mPFC projecting to the basal ganglia via the ventral striatum. We conducted a series of experiments to investigate neuroarchitecture of mPFC neurons projecting to the nucleus accumbens (NAc) and GABAergic medium spiny neurons (MSNs) projecting to the ventral midbrain (VM; i.e. ventral tegmental area and the medial substantia nigra) and how these pathways contribute to mPFC mediated anticipatory motivation. To address this question, we used optogenetics, brain slice electrophysiology, fiber photometry and intracranial self-stimulation in mice. **The mPFC-to-NAc pathway:** We found that while contralateral injections of the glutamate antagonists CNQX/AP5 into the NAc reduced the rates of ICSS rewarded with mPFC stimulation by 15% compared to vehicle injections, ipsilateral intra-NAc CNQX/AP5 injections reduced them by 74%, suggesting that glutamate transmission from mPFC neurons to NAc neurons plays an important role in mPFC reward. **The NAc-to-VM pathway:** We found that mice learned to seek for the stimulation of NAc^{MSNs} projecting to the VM. We also found that the stimulation of NAc^{MSNs} axonal projections in the VM evoked EPSCs in 15 of the 35 DA cells recorded using brain slice electrophysiology, and bath application of GABA receptor antagonists did not affect the evoked EPSCs. We then found that bath application of the NK1 receptor antagonist SR140333 (1 uM), but not the NK3 receptor antagonist SR142801 (1 uM), significantly reduced the magnitude of light-evoked EPSCs. We

are currently examining whether NK1 transmission mediates reward elicited by the stimulation NAc^{MSNs} projecting to the VM. **The mPFC-to-NAc-to-VM pathway:** We found that stimulation of both mPFC and mPFC axonal projections in the NAc activated VM DA neurons, using fiber photometry. Brain slice electrophysiology recordings revealed that a single 3-ms light pulse evoked excitatory postsynaptic potentials in NAc^{MSNs} projecting to the VM with a mean latency of 1.25 ms. Moreover, the effects were completely blocked by the presence of the AMPA receptor antagonist DNQX, suggesting that mPFC neurons monosynaptically excites NAc^{MSNs} projecting to the VM. Together, these experiments begin to show that mPFC stimulation elicits anticipatory motivation via the activation of NAc-to-VM neurons that, in turn, activates DA neurons through tachykinin transmission.

Disclosures: **A. Badawi:** None. **C. Yang:** None. **C.T. Potter:** None. **R.F. Don:** None. **A. Kesner:** None. **L.R. Whitaker:** None. **A. Kisner:** None. **S. Junn:** None. **Y. Aponte:** None. **S. Ikemoto:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.06/U36

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R00DA041350-02

Title: Role of afferent projections to dorsolateral striatum in incubation of methamphetamine craving in male and female rats

Authors: *I. R. DAVIS, S. A. COLDREN, X. LI;
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Abstract: Methamphetamine (Meth) seeking progressively increases after withdrawal from drug self-administration (incubation of Meth craving). We have previously shown that both dorsomedial and dorsolateral striatum (DMS and DLS) play critical roles in this incubation. Moreover, our recent anatomical tracing study examines afferent projections into DMS and demonstrates a novel role of projections from anterior intralaminar nucleus of thalamus (AIT) to DMS in incubation of Meth craving in male rats. Here we extend our previous study by investigating critical afferent projections into DLS in incubation of Meth craving in both male and female rats. We trained both male and female rats to self-administer Meth (6-h/d for 10 d). On withdrawal day 12, we injected cholera toxin subunit B (CTb, a retrograde tracer) unilaterally into DLS. On withdrawal day 26, we tested rats for relapse to Meth seeking and processed the brain for immunohistochemistry to double label CTb and Fos (a neuronal activity marker). We found no sex differences at the behavioral level and both male and female rats exhibited time-

dependent increase of Meth seeking after prolonged withdrawal. Ongoing studies are focusing on glutamatergic projections from insula cortex (a brain area previously implicated in Meth relapse after voluntary abstinence) and AIT. Future studies will use chemogenetic approaches to examine causal roles of these glutamatergic projections into DLS in incubation of Meth craving.

Disclosures: I.R. Davis: None. **S.A. Coldren:** None. **X. Li:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.07/U37

Topic: G.08. Drugs of Abuse and Addiction

Title: Dorsal raphe projections regulate the rewarding nature of amphetamine

Authors: D. N. TAPP, J. C. PERKINS, *M. S. MCMURRAY;
Psychology, Miami Univ., Oxford, OH

Abstract: Amphetamine's rewarding effects are thought to be driven primarily by its facilitation of dopamine release from dopamine projections to the ventral striatum. Normally, serotonin is known to modulate dopamine release, and may thus also control the rewarding effects of amphetamine. For example, co-administration of SSRIs has been found to significantly increase amphetamine-induced dopamine release and mesolimbic circuit activation; however, the specific neural circuit underlying this interaction remains unclear. Recent work has shown that activation of projections from the dorsal raphe nucleus (DRN) to the ventral tegmental area (VTA) increases mesolimbic dopamine release and can cause a conditioned place preference. Further, the projections between the DRN and the nucleus accumbens (NaC) are known to exist, but have not yet been behaviorally profiled. The purpose of this study is thus to determine which of these two circuits (DRN to VTA and/or DRN to NAc) modulates the rewarding nature of amphetamine. To accomplish this, we selectively increased activity in each circuit using chemogenetics (DREADDs). First, we determined if activation of each of these circuits increased amphetamine-induced neural activity in the striatum, as measured by the early immediate gene c-Fos. Next, we determined if increasing activity in these circuits can strengthen a conditioned place preference (CPP) for low-dose amphetamine, which generates only a weak place preference on its own. Preliminary results suggest that activation of DRN to VTA projections causes an enhancement of the rewarding nature of amphetamine, while activation of DRN to NAc projections decreases the rewarding nature of amphetamine. We hypothesize that these behavioral and neurological results are due to increased amphetamine-induced dopamine release via the DRN-VTA projections and a decrease in striatal activation by DRN-NAc projections. This study will enhance our understanding of the basic neural processes that control reward and

alter pharmacological mechanisms of commonly co-prescribed drugs, and will shed light on our understanding of individual differences in the rewarding nature of psychostimulants.

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Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.08/U38

Topic: G.08. Drugs of Abuse and Addiction

Support: MRC Programme grant (G1002231)
MRC Doctoral Training Partnership
E.G Fearnsides
Raymond & Beverly Sackler Fund

Title: Neurobehavioral analysis of addiction-relevant behavioural endophenotypes

Authors: *J. A. JONES[1], B. JUPP[1], S. J. SAWIAK[2], P. ZHUKOVSKY[1], M. A. KHAN[1], A. BELIN-RAUSCENT[1], M. FOUYSSAC[1], A. L. MILTON[1], B. J. EVERITT[1], T. W. ROBBINS[3], D. BELIN[1], J. W. DALLEY[1];
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Abstract: Considerable evidence highlights several behavioural vulnerability traits associated with cocaine use disorder, including impulsivity, anxiety, novelty-/sensation seeking and aberrant attribution of incentive salience to conditioned cues. Such traits have been operationalised and translated in experimental approaches in non-human animals and are associated with distinct behavioural features of drug addiction. Using 9.4T magnetic resonance imaging we investigated the functional and behavioural correlates of rats after assessment for several addiction-related endophenotypes across development, during adulthood, and prior to the assessment of compulsive cocaine seeking. Male Lister-Hooded rats were scanned on post-natal day 21, 35 and 63 and were subsequently screened for impulsivity (5-choice task), anxiety (elevated plus maze), appetitive conditioning (sign-/goal-tracking), novelty reactivity (locomotor activity) and novelty preference (novelty versus familiarity). We found that impulsivity, anxiety, sign-/goal-tracking and locomotor reactivity were each independent behavioural constructs (anxiety x impulsivity: r_s = -0.032, p=0.859, anxiety x sign-tracking: r_s = -0.006, p=0.975, anxiety x locomotor reactivity: r= -0.055, p=0.755, impulsivity x sign-tracking: r_s = -0.202, p=0.251, impulsivity x locomotor reactivity: r_s = 0.183, p=0.302, sign-tracking x locomotor reactivity: r_s = -0.156, p=0.377). Using resting-state functional MRI we found that functional connectivity strength between the hippocampus, ventral striatum and lateral orbitofrontal cortex

was differentially modulated in high- and low-impulsive rats. Such abnormalities may be linked to the increased propensity of high-impulsive rats to develop compulsive cocaine seeking. By distinguishing differences in functional connectomes of distinct behavioural traits this research has the potential to identify predictive biomarkers associated with individual risk for cocaine use disorder.

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Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.09/U39

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01EB022015

Title: Real time pharmacokinetic and pharmacodynamic measurements of drugs within the brains of freely behaving rats

Authors: *K. L. PLOENSE[1], P. DAUPHIN-DUCHARME[1], N. ARROYO-CURRAS[3], S. WILLIAMS[1], N. SCHWARZ[1], T. E. KIPPIN[2], K. W. PLAXCO[1];
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Abstract: The drug cocaine evokes complex neurochemical responses that mediate their influence of neural circuits underlying behavioral control, such as the dopamine pathways that we now know drive reward anticipation and locomotor behavior. In contrast, the anesthetic drug procaine functions as both a local anesthetic in the periphery, and a general anesthetic when it reaches the brain, affecting breathing, heart rate, and locomotion. However, the time resolution with which existing methods can measure these drugs is *orders of magnitude* poorer than the resolution with which physiological responses to these drugs occur. In response to this problem, our group has recently developed a new technology, electrochemical aptamer-based sensors (E-AB sensors), which are the size of a human hair and can detect physiologically relevant concentrations of specific drugs *in-situ*, in awake, freely behaving animals. Here, we tested this new technology on rats that have been administered either cocaine or procaine intravenously and measured the concentrations of these drugs within the lateral ventricle concurrent with monitoring locomotor activity. We successfully measured the concentration of cocaine and procaine every 10 s for 2 hours and generated a full pharmacokinetic and pharmacodynamic

profile for these drugs within the lateral ventricle. As cocaine concentrations increased (from 0 to 5 μM), they directly corresponded with increased locomotor activity and stereotypy, whereas increased levels of procaine in the lateral ventricle coincided with decreased heart rate, oxygen saturation (SPO2), and locomotion. In conclusion, we have developed a novel, potentially revolutionary, technology that can measure the pharmacokinetics and pharmacodynamics of psychoactive drugs within the brains of awake, freely behaving animals.

Disclosures: K.L. Ploense: None. **P. Dauphin-Ducharme:** None. **S. Williams:** None. **N. Schwarz:** None. **T.E. Kippin:** None. **K.W. Plaxco:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Diagnostic Biochips. **N. Arroyo-Curras:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.10/U40

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA021955

Title: The mTOR inhibitor everolimus blocks incubated cocaine-craving via reversing withdrawal-induced proteomic adaptations within prelimbic cortex

Authors: A. S. CHIU, E. K. SHULMAN, M. C. KANG, G. SHAB, K. N. ELIAS, A. M. FABELLA, K. N. HOLDER, B. D. BARGER, M. SANKARAN, T. E. KIPPIN, ***K. K. SZUMLINSKI**;
Psychological and Brain Sci. and the Neurosci. Res. Inst., Univ. California-Santa Barbara, Santa Barbara, CA

Abstract: A time-dependent increase in PI3K/Akt/mTOR signaling, concomitant with a reduction in mGlu1/5 expression, within the ventromedial prefrontal cortex (vmPFC) is critical for the incubation of cocaine-craving and/or perseverative drug-seeking behavior during protracted withdrawal. Herein, immunoblotting was conducted to localize cocaine withdrawal-induced changes in protein expression within the infralimbic (IL) and prelimbic (PL) subregions of the vmPFC and a series of behavioral studies examined the effects of pretreatment with the FDA-approved mTOR inhibitor Everolimus (formerly RAD 001; a.k.a., Zortress, Certican, Afinitor, Votubia, Evertor) upon cocaine-seeking in a rat model of intravenous cocaine self-administration. Rats trained to self-administer cocaine under 2 different short-access paradigms both exhibited incubated cocaine-craving that was accompanied by time-dependent increases in PL expression of phospho-Akt and Homer2, and decreases in PL expression of mGlu1/5. No or less robust cocaine-induced changes in protein expression were observed within the IL. Thus,

withdrawal from a history of short-access cocaine self-administration is sufficient to produce behavioral and proteomic adaptations within the PL akin to those reported within the vmPFC of rats with a history of long-access cocaine self-administration. Single, oral dosing with Everolimus (0, 0.01, 0.1 and 1.0 mg/kg), administered prior to behavioral testing, dose-dependently blocked the incubation of cocaine-seeking and this effect persisted for at least 24 h. Pretreatment with the maximally effective Everolimus dose (1.0 mg/kg) did not alter sucrose-seeking behavior nor did it impact cocaine-seeking behavior in rats tested in early withdrawal. Interestingly, 1.0 mg/kg Everolimus treatment, administered following a test for incubated cocaine-seeking, also reduced subsequent responding, arguing a facilitation of the consolidation of extinction memory. The "anti-incubation" effect of 1.0 mg/kg Everolimus was associated with a reversal of withdrawal-induced increases in Akt activity, as well as Homer2a/b levels, in addition to a complete reversal of the decrease in mGlu1/5 expression within the PL. These data provide novel, exciting, evidence that acute oral Everolimus pretreatment blocks the expression of incubated cocaine-seeking in a rat model of cocaine-craving and does so by reversing withdrawal-induced proteomic adaptations within the PL that drive cue super-reactivity. Such findings provide preclinical evidence for repurposing FDA-approved mTOR inhibitors for interrupting cocaine-craving during protracted withdrawal.

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Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.11/V1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01EB022015

Title: A novel method for real time, high-precision, in brain measurements of serotonin using electrochemical aptamer-based (E-AB) biosensors

Authors: ***J. GERSON**[1], K. PLOENSE[3], K. PLAXCO[2], T. E. KIPPIN[4];
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Abstract: Serotonin (5-HT) is a neurotransmitter that has critical participation in a wide range brain functions, such as arousal, motivation, learning, etc, and equally has been implicated in disorders, such as depression, anxiety, schizophrenia, addiction, insomnia e.t.c. Despite its implication in almost all forms of psychiatric disorders, the ability to study serotonin levels in

brain has seen extremely limited evolution over the last 50 years and pales in comparison to our ability to resolve changes in neurotransmitters such as dopamine. Consequently, real-time and high temporal resolution, in-brain measurements of 5-HT would be extremely helpful in characterizing 5-HT changes in normal brain function, as well as characterizing deviations that may arise as a result of disorders proposed to involve 5-HT. Our group has created and implemented the electrochemical aptamer-based biosensor (E-AB) platform to enable the detection of small molecules in living subjects both in real time and with high temporal (sub-second) resolution. Here, we describe our development of an E-AB sensor capable of detecting physiologically relevant concentrations (low nM - high uM, Kd = 500nM) of 5-HT in vitro settings (artificial and fetal bovine CSF). Modification of a previously reported optical aptamer, selective for 5-HT, allows us to attach the aptamer to a gold working electrode surface and, through the use of a redox reporter, translate changes in current to concentration levels. Currently, we are testing the utility of this E-AB sensor to measure 5-HT release in a variety of brain regions in response to administration of exogenous drugs, by using a specially designed micro-probe designed to function in freely moving animal subjects. If successful, this research program is positioned to transform our understanding of 5HT dynamics in the same way that cyclic voltammetry has transformed our understanding of dopamine system function and the plethora of behavioral outputs it is associated with.

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Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.12/V2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA grant R01DA039168

Title: Effects of manipulating PFC-NAC sub-circuit activity on methamphetamine use in B6 mice

Authors: *C. N. BROWN, J. BELTRAN, W. YEN, T. TRAN, B. BARGER, N. WILLIAMS, A. PARK, T. KIPPIN, K. SZUMLINSKI;
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Abstract: Prior research on the neurobiology of methamphetamine (MA) use has primarily probed the effects of high MA doses that model late-stage addiction. Not so well characterized are the neurological changes during early stages of drug use that may help to explain individual variation in vulnerability to MA addiction and/or the transition from recreational use to addiction. The long-term neuroplasticity underlying changes over time to dose-dependent

subjective MA effects has been strongly associated with glutamate signaling, particularly the glutamatergic projection from the prefrontal cortex (PFC) to the nucleus accumbens (NAC). Sub-circuits of this projection travel from the prelimbic (PL) PFC, which is thought to drive reinforced behaviors, to the core of the NAC, which is thought to contribute to decision-making by encoding the motivational value of expected goals, and the shell of the NAC, which is known for updating reward saliency following new experiences. Activity of these sub-circuits were manipulated bidirectionally using a dual virus chemogenetic approach called Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). A retrograde adeno-associated viral vector (AAV) carrying Cre was infused into the NAC core or shell, and then an AAV carrying either a Cre-dependent excitatory DREADD, Cre-dependent inhibitory DREADD, or mCherry control was infused into the PL, ensuring that only neurons projecting from the PL to the core or shell would express the DREADDs or mCherry. Operant conditioning procedures were utilized to assess the effects of these manipulations on the acquisition of MA use in male B6 mice. Activating the PL-shell sub-circuit significantly reduced the number of mice that reached acquisition criteria, as well as the amount of active responses, during the acquisition phase, compared to the control or inhibitory viral infusions. Manipulating the PL-core sub-circuit revealed no statistically significant effect of the viral infusions. These results may indicate that the NAC shell sub-region is more involved with MA acquisition, which reflects its role in updating decision making with new experiences. The core may be more responsible for guiding decision-making following drug experience, which should be explored in future studies probing the effects of manipulating its sub-circuits on the behavior of drug-experienced mice.

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Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

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Program #/Poster #: 075.13/V3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 5R01AA024044-04
NSF GRFP Fellowship

Title: Both male and female mice exhibit comparable age-related differences in the incubation of alcohol withdrawal-induced negative affect: A study of molecular correlate

Authors: *C. L. JIMENEZ CHAVEZ, L. W. BREWIN, A. LAGUNA, M. A. COELHO, D. LIEBERMAN, I. SWAUNCY, T. TRAN, T. ALBANESE, I. GABRIELLA, S. L. SCUDDER, K. K. SZUMLINSKI;
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Abstract: In male C57BL/6J mice, the manifestation of alcohol withdrawal-induced negative affect following a history of binge-drinking is associated with increased indices of mGlu/Homer2-related signaling within extended amygdala structures, and varies as a function of the age of binge-drinking-onset. The present study examined for potential sex differences in the age-dependent manifestation of negative affect during early (1-day) and protracted (70 days) withdrawal from binge-drinking (2 h/day for 14 days; simultaneous presentation of 5, 10, 20 and 40% v/v) and related group differences in negative affect to plasma corticosterone and protein indices of mGlu/Homer2 signaling within the shell and core subregions of the nucleus accumbens (NAs and NAc, respectively). As expected, adolescent mice consumed more alcohol than adults and female mice consumed more alcohol than males. Both male and female mice with a history of binge-drinking during adulthood (PND56-70) exhibited greater signs of negative affect on marble-burying and forced swim tests during early withdrawal than adolescent animals and this age-related difference was associated with higher basal plasma corticosterone levels and higher Homer2a/b expression within the NACs, but not NACc. In both male and female mice with a history of binge-drinking during adolescence, the manifestation of negative affect incubated during protracted withdrawal, concomitant with a rise in basal plasma corticosterone. Interestingly, basal corticosterone levels remained elevated in adult female binge-drinkers at the later time-point, despite a normalization of affective behavior. These findings provide evidence that the manifestation of alcohol withdrawal-induced negative affect, and corresponding changes in basal corticosterone, follows a comparable temporal profile in male and female mice, irrespective of the age of drinking onset. Further, these data provide additional support for a role for Homer2 expression in age-related differences in the negative affective consequences of binge-drinking.

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Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

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Program #/Poster #: 075.14/V4

Topic: G.02. Motivation

Support: DA035217
MH101146

Title: Role of midbrain mTOR signaling in hyperactivity and attention deficit behaviors

Authors: *X. LIU, C. VICKSTROM, M.-M. HU, Y. HU, Q.-S. LIU;
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Abstract: Attention-deficit/hyperactivity disorder (ADHD) is a highly prevalent neuropsychiatric disorder in children. Many genetic risk factors associated with ADHD are variants in genes involved in dopaminergic neurotransmission. Mechanistic target of rapamycin (mTOR) is a serine/threonine-protein kinase that regulates cell growth and proliferation by controlling protein synthesis. We generated dopamine neuron-specific mTOR conditional knockout (cKO) mice by crossing mTOR-loxP/loxP mice with dopamine transporter-Cre (DAT-Cre) mice. We found that the size of dopamine neurons was significantly decreased in DAT-mTOR-cKO mice compared with that of wild-type controls. In the open field test, DAT-mTOR-cKO mice exhibited a pronounced (~2 fold) increase in basal locomotor activity compared with that of wild-type mice, and intraperitoneal injection of cocaine or amphetamine markedly augmented locomotor activity in wild-type mice but did not produce a stimulant effect in DAT-mTOR-cKO mice. Additionally, DAT-mTOR-cKO mice exhibited inattention, with impairments of recognition learning in the olfactory habituation test and the novel object recognition test compared with wild-type controls. We also found that DAT-mTOR-cKO mice showed decreased evoked dopamine release and prolonged dopamine reuptake in the nucleus accumbens. Taken together, we revealed that mTOR signaling in dopamine neurons plays a critical role in regulating ADHD-like behaviors.

Disclosures: **X. Liu:** None. **C. Vickstrom:** None. **M. Hu:** None. **Y. Hu:** None. **Q. Liu:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.15/V5

Topic: G.02. Motivation

Support: NIH AA022506

Title: Sex and strain differences in preference for a context associated with voluntary activity

Authors: K. M. BORDASH, K. T. LEONARD, C. A. DAVIS, ***J. E. G. GRISEL**;
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Abstract: Individual differences in the propensity to engage in voluntary activity are mediated by a host of biological, social and cultural factors, including genetic and hormonal influences. Sex differences in voluntary activity are well established in humans and other animals. With the notable exception of humans, females are more active than males, however the mechanisms for these sex differences are unknown. In particular, whether or not this disparity reflects sex differences in either the positive or negative reinforcing effects of voluntary activity is an open question with potentially important clinical implications. Although previous research has employed a conditioned place preference (CPP) paradigm to evaluate the rewarding effects of

voluntary exercise (e.g., *Iverson,* 1993) to our knowledge this has only been investigated in male subjects. To address this gap in the literature we assessed CPP to a context associated with access to a rotating running wheel compared to an alternative context associated with access to a static wheel, in male and female, DBA/2J and C57BL/6J mice. On Day 1, subjects had free access to a three chambered conditioning arena. The central chamber was stimulus-neutral but the two conditioning contexts varied in terms of floor texture. On days 2, 4 and 6 subjects were left undisturbed in their group home cages. On Day 3 and 5, the mice were conditioned for 60 minutes total, 10 minutes without the wheel and 50 minutes with the wheel (either free or locked) in the chamber. Free wheels were associated with the non-preferred context in a biased design. A 20 minute preference testing period occurred on Day 7,with no activity wheels present. Only females showed a preference for the rotating wheel associated context, with preference in DBA/2J greater than in C57BL/6J. This model enables assessment of the rewarding effects of wheel running by observing the overall relationship between preference and voluntary activity, and is sensitive to sex and strain influences.

Disclosures: J.E.G. Grisel: None. **K.M. Bordash:** None. **K.T. Leonard:** None. **C.A. Davis:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.16/V6

Topic: G.02. Motivation

Support: R01 AA013983
F31 AA025827
R01 DA031734

Title: Modeling pathological aggressive motivation: VTA CRF circuits in aggression-specific arousal

Authors: H. E. COVINGTON, III, K. CHIEN-YOUNG, E. L. NEWMAN, N. AKDILEK, K. HA, L. CART, E. SINGLETON, M. Z. LEONARD, ***K. A. MICZEK**;
Tufts Univ., Medford, MA

Abstract: Phylogenetically conserved mammalian aggression can serve as a reinforcer and can become excessive. Stereotypical “scalloped” patterns of fixed interval (FI) responding - a rapid acceleration of responding as the end of the interval approaches - is a sensitive way to measure one’s underlying motivational state when they are anticipating their attack. C57 mice will respond in this predictable manner when reinforced by the opportunity to fight, and by interactions with a sexual partner, indicating that these social stimuli both serve as potent

positive reinforcers. The stress neuropeptide corticotropin-releasing factor (CRF) is released into the ventral tegmental area (VTA) when a reward is anticipated. Indeed, CRFR1 receptors were found to share a general role in the motivation to seek social (e.g., aggression, psychosexual) and other natural rewards (e.g., saccharine, running wheel access). However, a selective role for CRFR1 receptors in the VTA appears to be particularly important for the motivation to fight, which can be dissociated from subsequent fighting performance. In addition, CRF in the VTA modulates dopamine release in the ventral striatum during the anticipation of a fighting reward. In sum, FI schedules can be reliable tools for identifying the neurobiological mechanisms that are involved in the anticipation, but not consumption, of natural and excessive rewards. This ethological approach links the selective neural actions of neuropeptides like CRF in the VTA to the propagation of maladaptive violent behaviors.

Disclosures: K.A. Miczek: None. **H.E. Covington:** None. **K. Chien-Young:** None. **E.L. Newman:** None. **N. Akdilek:** None. **K. Ha:** None. **L. Cart:** None. **E. Singleton:** None. **M.Z. Leonard:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.17/V7

Topic: G.02. Motivation

Support: R01 AA013983
F31 AA025827

Title: Escalation of aggressive motivation after rapid alcohol self-administration: Role of CRF

Authors: *H. E. COVINGTON, III, N. AKDILEK, E. L. NEWMAN, W. HAYEK, L. CART, R. GENEROSO, K. CHIEN-YOUNG, K. HAZZARD, C. LI, K. A. MICZEK;
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Abstract: Alcohol drinking in some individuals can escalate violent behaviors. The present series of experiments determined how alcohol, when self-administered in C57 mice, escalates (1) the motivation to fight, or (2) the execution of fighting performance, when using a novel experimental chamber that accommodates chain-scheduled access to multiple reinforcers, including alcohol and social rewards. Specifically, we sought to examine if daily access to alcohol self-administration would alter the motivation to fight, and whether this change occurs with, or without, shifts in the performance of fighting behavior. Responding for alcohol reinforcements (20% EtOH diluted in SuperSac) under the control of a simple schedule of reinforcement (fixed ratio, FR1) was chained to a short (five-minute) fixed interval (FI5) schedule for an aggression reward. This chain schedule was implemented for at least 21

consecutive days. All mice reliably self-administered alcohol during each daily session, but only a small percentage rapidly consumed higher than 1.5 g/kg at least 7 times throughout the 21 days of access. These binge-like drinkers produced accelerated rates of responding for the opportunity to fight, while their fighting performance remained disrupted by alcohol. As compared to non-bingers, average daily alcohol intake also increased in these mice over the 21 days of access. In separate experiments, we confirmed that repeated oral administrations of either 1.8 or 2.2 g/kg alcohol (PO) sensitize FI rates of responding for aggression rewards, but not for psychosexual, running-wheel access, or saccharine rewards. Furthermore, the expression of escalated motivation for aggression rewards depends on the actions of corticotrophin releasing hormone (CRH), as CRH R1 antagonism reduced levels of alcohol-escalated responding. Under these conditions, alcohol self-administration engenders neural and behavioral plasticity that specifically enhances the arousal associated with aggression-seeking.

Disclosures: H.E. Covington: None. **N. Akdilek:** None. **E.L. Newman:** None. **L. Cart:** None. **R. Generoso:** None. **K. Chien-Young:** None. **K. Hazzard:** None. **C. Li:** None. **K.A. Miczek:** None. **W. Hayek:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.18/V8

Topic: G.08. Drugs of Abuse and Addiction

Title: The connectome of the dopamine neuron in the addicted brain

Authors: *G. WILDENBERG[1], J. KORANDA[2], X. ZHUANG[3], N. B. KASTHURI[4]; [1]Neurobio., Univ. of Chicago/Argonne Natl. Lab., Chicago, IL; [2]Neurobio., [3]Dept Neurobiol, Univ. of Chicago Dept. of Neurobio., Chicago, IL; [4]Neurobio., Univ. of Chicago, Chicago, IL

Abstract: The ways in which addiction changes neural circuitry are poorly understood. Uncovering these changes would provide new mechanisms for explaining addictive behaviors and future targets for therapeutic intervention. We combine recent advances in protein engineering of peroxidases for labeling for electron microscopy (e.g. APEX2) with large volume serial electron microscopy (EM) to reconstruct how the connectivity of dopaminergic neurons changes in the brains of mice addicted to cocaine. Specifically, we inject AAV expressing cre-dependent APEX2 in transgenic animals expressing Cre in dopamine neurons (e.g. DAT-Cre animals). Mice were administered 5 daily intraperitoneal injections of either saline or cocaine and sacrificed 2 days following the last injection. We reconstructed the dendrites of APEX2 labeled DA neurons in the Ventral Tegmental Area (VTA) and axons in the Nucleus Accumbens (NA) (~0.5 mm x 0.5mm x 0.2mm volumes). Preliminary data suggests that dopaminergic axons in the NA are more branched than control DA axons and that many of the axonal branches are

non-synaptic and instead terminate in large mitochondria filled ‘bulbs’ reminiscent of retraction bulbs found in the developing and the damaged brain. These novel findings opens up future applications for tracking the role structural changes in neural circuitry underlying addictive behavior.

Disclosures: G. Wildenberg: None. **J. Koranda:** None. **X. Zhuang:** None. **N.B. Kasthuri:** None.

Poster

075. Neural Mechanisms Underlying Motivated Behaviors and Addiction

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 075.19/V9

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA041694-01
NIH Grant DA09397

Title: Laterodorsal tegmental nucleus glutamate inputs to the ventral tegmental area are critical for the development of cocaine locomotor sensitization in mice

Authors: A. PURANIK[1], D. ARIZANOVSKA[1], C. BULLARD[1], N. BUIE[1], P. VEZINA[3], ***S. STEIDL**[2];
[1]Psychology, [2]Loyola Univ. Chicago, Chicago, IL; [3]Dept Psychiatry and Behavioral Neurosci., The Univ. of Chicago, Chicago, IL

Abstract: Drug-induced elevations of forebrain dopamine (DA) are critically related to their reinforcing and habit-forming effects. Sensitization – an enhancement in the ability of drugs of abuse to activate DA neurotransmission, increase locomotion and support self-administration following previous drug exposure – is thought to reflect underlying neural changes important for drug addiction. Drug-induced potentiation of ventral tegmental area (VTA) glutamate (GLU) signaling and changes in GLU receptors critically contribute to the induction of sensitization. We used optogenetic techniques to test the role of laterodorsal tegmental nucleus (LDTg) GLU neurons, one of several sources of VTA GLU input, in the development of cocaine locomotor sensitization. Halorhodopsin (NpHR) or a control viral vector (eYFP) was expressed in LDTg GLU neurons of VGluT2::Cre mice. Separate groups of NpHR and eYFP mice were implanted with bilateral optic probes aimed at either the LDTg or the VTA. Mice were then injected with either cocaine (COC, 15 mg/kg; i.p.) or saline (SAL; 10 ml/kg; i.p.) for five consecutive days and locomotion was measured for 1 hour on each day. During each of these sessions light stimulation (532 nM, 5-8 mW, continuous) was provided into either the LDTg or the VTA to inhibit LDTg GLU cell bodies or their VTA afferents, respectively. One week later all mice received a COC challenge injection (15 mg/kg; i.p.) in the absence of light stimulation. In eYFP

control mice locomotion was increased following COC pretreatment relative to SAL pretreatment. By contrast, inhibition of either LDTg GLU cell bodies or their VTA afferents during the induction phase blocked the subsequent expression of locomotor sensitization. These data identify the LDTg as the critical source of VTA GLU for the development of locomotor sensitization. The LDTg may thus give rise to the VTA GLU synapses at which the GLU plasticity, known to contribute to the enhancement of addictive behaviors, occurs.

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Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.01/V10

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: The Strategic Research Program for Brain Sciences from Japan Agency for Medical Research and Development, 579
The Grant-in-Aid for Scientific Research on Innovative Areas from the MEXT, 16H06462

Title: Enhanced anxiety-like behaviors by lactate dehydrogenase inhibitor treatment in a mouse model of chronic social defeat stress

Authors: *H. HAGIHARA, H. SHOJI, Y. TAKAMIYA, T. MIYAKAWA;
Fujita Hlth. University, ICMS, Div. Syst. Med., Toyoake, Japan

Abstract: Exogenous treatment with lactate has been found to have antidepressant effects through studies on animal models of stress, such as corticosterone treatment model and social defeat stress model, while increased lactate levels have been found in the brain of patients with depression and anxiety disorders, and the corresponding animal models. Here, we investigated the role of endogenous lactate production in response to stress on anxiety and depression-like behavior in a mouse model of social defeat stress. We first confirmed that lactate levels in the brain increase immediately after a single exposure to defeat stress, and then attenuated these levels by prior treatment with sodium oxamate (OX), a lactate dehydrogenase inhibitor that blocks lactate production. We then conducted a chronic experiment in which mice experienced daily defeat stress for ten days, and thereafter, were processed for behavioral tests. Mice were treated with OX or vehicle before each defeat session. There were no differences in susceptibility to the repeated defeat stress, as assessed by social avoidance test, between defeated mice with OX and vehicle treatment. However, we found that anxiety-like behavior, as assessed by the open field test and light-dark transition test was increased in OX-treated, defeated mice

compared to vehicle-treated, defeated mice. Treatment with OX did not affect depression-like behavior, as assessed by the Porsolt forced swim test and tail suspension test, in defeated mice. Following the behavioral tests, whole brains were harvested and processed for lactate measurement. Results showed that when compared with control mice, chronic defeat stress increased brain lactate levels in vehicle-treated mice and to a large extent in OX-treated mice, when focused on the mice with susceptibility to stress. Mice chronically treated with only OX did not show such enhanced anxiety-like behavior or increased lactate levels in the brain. The results suggest that repeated inhibition of acute lactate production in response to defeat stress leads to its sustained elevation and enhanced anxiety-like behavior in chronic phase.

Disclosures: H. Hagihara: None. **H. Shoji:** None. **Y. Takamiya:** None. **T. Miyakawa:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.02/V11

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Fellowship/ Programa de Doutorado Sanduíche no Exterior (PDSE)/Process no. 88881.187105/2018-01
NIH DA022340

Title: Chronic social defeat stress promotes changes in motivated behavior and mesolimbic dopamine function that are limited to social contexts

Authors: *C. A. FAVORETTO[1], D. P. COVEY[2], M. E. FOX[2], I. M. H. QUADROS[1], M. K. LOBO[2], J. F. CHEER[2];
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Abstract: Chronic stress is a primary factor that promotes persistent depressive symptoms in both humans and animal models. In rodents, repeated exposure to chronic social defeat stress (CSDS) promotes depressive-like behavior, such as social avoidance and anhedonia. CSDS also leads to functional changes in the dopaminergic mesolimbic pathway that underlie, at least partially, the depressive-like behavioral impairments. However, depression is multifaceted and a prominent symptom in many individuals is decreased motivational drive to seek rewards, despite intact hedonic processing. Because mesolimbic dopamine projections are central to reward processing and motivation, we predicted that CSDS-induced changes in dopamine function generalize to non-social stimuli and promote altered goal-directed behavior. To test this, male C57BL/6J mice were subjected to 10 days of social defeat and then evaluated during social

interaction and sucrose reinforcement while monitoring dopamine release in the nucleus accumbens using fast-scan cyclic voltammetry (FSCV). In line with prior work, CSDS promoted social avoidance and an increase in dopamine release events during exposure to a conspecific male in a social interaction test. However, we found no change in motivation for a non-social reinforcer or dopamine release during operant responding for sucrose pellets on a progressive-ratio schedule. We found that CSDS suppressed social interaction and increased accumbal dopamine release to a conspecific, but did not promote general depressive-like symptoms. Thus, CSDS supports behavioral and dopaminergic adaptations that are specific to social contexts.

Disclosures: C.A. Favoretto: None. **D.P. Covey:** None. **M.E. Fox:** None. **I.M.H. Quadros:** None. **M.K. Lobo:** None. **J.F. Cheer:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.03/V12

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIMH Grant R01 MH112861

Title: Social instability stress and chronic non-discriminatory social defeat are effective chronic stress paradigms for both male and female mice

Authors: *C. N. YOHN[1], A. BAZER[1], S. ASHAMALLA[1], A. DIETERICH[2], B. A. SAMUELS[1];
[1]Psychology, Rutgers Univ., Piscataway, NJ; [2]Neurosci., Rutgers Univ., New Brunswick, NJ

Abstract: Despite stress-associated disorders having a higher incidence rate in females, historically preclinical research in rodents mainly utilizes males. Chronic stress paradigms, such as chronic social defeat and chronic corticosterone administration, were mainly designed and validated in males and subsequent attempts to use these paradigms in females has demonstrated sex differences in the behavioral and HPA axis response to these stressors. Here, we describe the behavioral and neuroendocrine response to two novel social stress paradigms, social instability stress (SIS) and chronic non-discriminatory social defeat stress (CNSDS). SIS exposes adult mice to unstable social hierarchies for 7 weeks. The CNSDS model simultaneously introduces male and female C57BL/6J mice into the home cage of resident CD-1 aggressors for 10 daily 5-minute sessions, with CD-1 aggressors attacking males and females indiscriminately. SIS effectively induces negative valence behaviors and hypothalamus-pituitary-adrenal (HPA) axis activation in both males and females. Additionally, the effects of SIS on negative valence behaviors are reversed by chronic antidepressant treatment with fluoxetine (FLX) in both males and females. In the CNSDS paradigm, stress resilient (RES) and susceptible (SUS)

subpopulations emerge in both sexes, with SUS mice displaying increased negative valence behaviors relative to RES and control mice in both sexes. Furthermore, SUS male and female mice displayed HPA axis activation following CNSDS exposure. Overall, these data demonstrate that the SIS and CNSDS paradigms are ethologically valid approaches to effectively induce chronic stress in both adult male and female mice.

Disclosures: C.N. Yohn: None. **A. Bazer:** None. **S. Ashamalla:** None. **B.A. Samuels:** None. **A. Dieterich:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.04/V13

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: CAPES
CNPq Grant 305500/2013-9

Title: Chronic unpredictable stress cause alterations in monoamine systems in the brain but leads to no behavior changes in rats

Authors: *J. ZACCARELLI-MAGALHÃES[1], G. R. ABREU[1], M. MANES[1], A. R. FUKUSHIMA[1], N. MOREIRA[1], E. L. RICCI[2], H. S. SPINOSA[1];
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Abstract: Depression is a complex multifactorial mental disorder that affects millions of people of all ages worldwide. The precise mechanism of the neurobiological processes of this disease is still unknown and to help elucidate such mechanism, several animal models have been developed. The chronic unpredictable stress (CUS) is a model that which exposes animals to a series of random mild stressors for a short period of time, leading to changes in behavioral and physiological parameters. Given the importance of the use of animal models for the understanding of the psychopathology of depression, more studies need to be conducted for the development of new models and improvement of old models. In this context, the aim of this study was to evaluate the behavioral and neurochemical changes caused by the CUS model in rats. Fourteen rats were subdivided into 2 groups, control and CUS, (n=7 animals/group). The CUS group was exposed to a random patter of mild stressors twice a day for a 10 days period, while the control group was handled only once a day for this period. A behavioral evaluation was performed using the sucrose preference, forced swim and light/dark box tests to measure changes that correspond to some symptoms presented by patients with depression. After the last behavioral test, all rats were euthanized by decapitation for collection of brain structures for

neurochemical analysis. The results of the behavioral evaluation did not showed alterations in any of the tests, indicating that all animals behave accordingly, regardless of the exposure of stressors, suggesting that this model did not reproduce the depressive phenotype in these animals, as previously described in the literature. Regarding the neurochemical evaluation, the analysis showed that 1) the CUS group had an increase in VMA levels (t= 0.0056) and in VMA/NOR turnover rates (t= 0.0020) in the hypothalamus; 2) the CUS group had an increase in NOR (t= 0.0244) and DOPAC (t= 0.0238) levels and a decrease in 5HIAA (t= 0.0159) levels in the hippocampus; and 3) the CUS group had an increase in NOR (t=0.0002) and DA (t= 0.0174) levels and a decrease in 5HT levels (t= 0.0007) and in VMA/NOR turnover rates (t= 0.0001) in the striatum. These results indicate that the CUS model did cause major alterations in the monoamine systems in different brain regions, similarly to the ones found in depressive patients, as describe in the literature. In conclusion, despite the lack of behavior alterations, the CUS model could help further studies to elucidate the neurobiological processes that cause depression.

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Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.05/V14

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: JSPS KAKENHI#19K17090
MSD
Pfizer

Title: Development of a mouse model of depression using emotional stress

Authors: *T. SEKI[1], H. YAMAGATA[1], A. KOBAYASHI[1], S. UCHIDA[2,1], Y. WATANABE[1,3], S. NAKAGAWA[1];
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Abstract: Background: The neurobiological mechanism of major depressive disorder (MDD) is still unclear. Therefore, it is difficult to develop an animal model that reproduces the pathophysiology of MDD faithfully. Stress is a well-known risk factor for the onset of MDD, and physical stressors, for example, strain stress or social defeat stress, have been used in animal models of depression to assess the depressive state. However, physical stress may militate against the depressive behavior of rodents. In this study, we investigated whether emotional

stress without physical stress could be used in a mouse model of depression.
Material and methods: We used BALB/c male mice, which reportedly have stress vulnerability. The emotional stress (ES) group witnessed social defeat stress through the wire netting for 7 days. The control group was reared without stress. Depression-like behaviors were evaluated using the open field and forced swim tests. We examined the expression of histone deacetylases in the brain, which were reported to be associated with the pathology of MDD.
Results and discussion:The ES group showed significantly more depression-like behaviors than the control group . The expression of histone deacetylases was significantly altered in the ES group compared to that in the control group. The ES model can be utilized as a new depression model for molecular biological research of depression in the future.

Disclosures: **T. Seki:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; kakenhi#19K17090. **H. Yamagata:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); research support from Pfizer and MSD. **A. Kobayashi:** None. **S. Uchida:** None. **Y. Watanabe:** None. **S. Nakagawa:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); research support from Pfizer and MSD.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.06/V15

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Korea basic research program through Korea Brain Research Instititue funded by Ministry of Science and ICT (19-BR-02-05)
Basic Science Research Program throught the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018M3C7A1024150)

Title: Impairment of episodic memory in an animal model of depression induced by chronic unpredictable stress

Authors: *J. KIM[1,3], J. LEE[4], T.-E. KIM[2,3], H. KANG[4], J. KOO[1,3];
[1]Korea Brain Res. Inst., daegu, Korea, Republic of; [2]Korea Brain Res. Inst., Daegu, Korea, Republic of; [3]DGIST, Daegu, Korea, Republic of; [4]Chung-Ang Univ., Seoul, Korea, Republic of

Abstract: Chronic stress occurs in everyday life and often results in depressive symptoms. Furthermore, clinical and preclinical studies indicated that cognitive dysfunction is related to major depressive disorder (MDD) (Raymond W Lam et al, 2014). Chronic unpredictable stress (CUS) is one of the animal behavior models for depression, which mimics daily-life stress in

humans. Previous studies showed that CUS affect cognitive function as well as mood regulation (CO Bondi et al, 2008). However, it remains unclear that CUS may induce non-declarative or declarative memory dysfunction. To investigate cognitive impairment in various memory forms in the animal depression model, we first exposed mice to 2-3 various stressors per day for 28 days (CUS). After CUS, we performed the forced swim test (FST) and sucrose preference test (SPT) to confirm induction of depressive-like phenotypes. Additionally, we performed three chamber sociability (or novelty) and elevated plus maze (EPM) tests to examine social dysfunction and anxiety-like behavior, respectively. Finally, we performed the WWW tasks (What Where When tasks) to investigate the CUS effect on episodic memory. CUS successfully not only induced depressive, anxiety-like behaviors in FST, SPT, EPM tests but also decreased social preference and novelty. In particular, we separated the CUS group into stress-susceptible and stress-resilient based on SPT data. The CUS-susceptible group showed significantly increased depressive, anxiety-like behaviors in FST, SPT, EPM, but CUS-resilient group did not. Also, the CUS-susceptible group displayed decreased social preference compared to CUS-resilient and CUS-control groups. Additionally, we performed the optogenetics in the medial prefrontal cortex (mPFC) which is well known to a hub brain region for depressive-like behavior. We found phasic stimulation of mPFC is sufficient sufficient for recovery ofed depressive-like behavior. Finally, we investigated whether CUS induce dysfunction of episodic memory using the WWW tasks, and found that the CUS group showed a significant decrease in all of the WWW tasks. These findings suggest that CUS, which causes depressive- and anxiety like-behaviors, can induce social and episodic memory dysfunction. Especially, the CUS-susceptible group showed dysfunction in emotional and social behaviors than compared to the CUS-resilient group. We have plans to investigate the impairment of episodic memory in the CUS-susceptible vs. CUS-resilient groups and the relevant neural circuits to the dysfunction of cognitive function in the CUS model.

Disclosures: **J. Kim:** None. **J. Lee:** None. **T. Kim:** None. **H. Kang:** None. **J. Koo:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.07/V16

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Funding was provided by The College of Health Sciences, University of KwaZulu-Natal

Title: Hippocampal 5HT1$_A$ and 5HTT alterations lead to cognitive deficits associated with major depressive disorder in a 14-day combined stress rat model

Authors: *G. T. NGOUPAYE[1], T. MADLALA[2], M. MABANDLA[2];
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Abstract: Current models used to study the pathophysiology of major depressive disorder (MDD) associated with cognitive deficits are laborious and time consuming. A novel 14-day combined stress model of depression associated with cognitive deficits was previously explored only in mice. This study, aimed first to establish the 14-day combined stress model in rats and to assess the involvement of the serotonin transporter (5HTT) and Serotonin 1A receptor (5HT1A) in the pathophysiology and further compare its mechanisms to a known 28- day corticosterone model. Adult Sprague Dawley male rats were subjected to a combined stress that included corticosterone (40mg/kg subcutaneously) injection and chronic immobilization stress for 2 hours daily for 14 days to induce depressive- like behaviour associated with cognitive deficit. The sucrose preference and Morris Water Maze tests were applied to test for depressive-like behaviours and cognitive abilities respectively. The hippocampus and prefrontal cortex (PFC) were harvested to assess acetylcholinesterase concentration using ELISA, and to assess the expression of 5HT1A, 5HTT proteins using PCR. Our findings showed that the 14-day and the 28-day groups consumed less sucrose solution than water. Animals from the 14-day group spent more time in the target quadrant and showed an increased level of acetylcholinesterase in the hippocampus while animals from the 28- day group showed an increased level in the prefrontal cortex. In addition, 14-day increased the 5HT1A in both the hippocampus and PFC and decreased 5HTT expression in the hippocampus. The 28-day group showed an increase of 5HT1A expression and a decreased 5HTT in the PFC.
Our results showed that 14-day induced depressive like behaviour with early cognitive deficit in rats with the hippocampus being the main structure affected. The 14-day combined stress model can therefore be useful in investigating new, comprehensive treatment strategy for MDD.
Key word: Combined stress, Depression, Cognitive deficit, Hippocampus, 5HT1A, 5HTT.

Disclosures: **G.T. Ngoupaye:** A. Employment/Salary (full or part-time):; University of Kwazulu-Natal, college of health science, School of laboratory, medicine and medical science. **T. Madlala:** None. **M. Mabandla:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.08/V17

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Fondo SEP-CINVESTAV #141

CONACyT #485970

Title: Behavioral characterization of the effect of chronic social defeat stress in Swiss Webster mice as a model of depression and cognitive impairment

Authors: *H. M. MANCHA-GUTIÉRREZ, C. LOPEZ-RUBALCAVA;
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Abstract: The early exposure to chronic stress has been proposed as a risk factor to the development of psychiatric disorders such as depression. Currently, the interaction between depression and cognitive impairments has become relevant because of its high comorbidity. The chronic social defeat stress (CSDS) is an animal model of depression, in which a susceptible adolescent male mouse (C57BL/6 strain) is exposed to physical encounters with another adult male mouse (CD1 strain) during 10-15 consecutive days. Traditionally, these strains have been used because of their greater susceptibility to stress and their high level of aggressiveness, respectively. We aimed to characterize the behavioral effects of the CSDS in male mice of the Swiss Webster (SW) mouse strain. First, we compared the behavioral effects of two protocols of CSDS (10 min/day for 15 days and 5 min/day for ten days) in adolescent male mice. Second, we evaluated if social avoidance behavior could be developed towards a mouse of the same age and strain. Third, we determined both long and short-term behavioral and cognitive effects of the CSDS (5 min/day for 10 days) in adolescent male mice. Finally, we determined the short-term behavioral effects induced by three different corticosterone (CORT) treatments (25, 50 or 100 µg/ml for 10 days). Results showed that both protocols were able to induce depression-like behaviors such as social avoidance and behavioral despair. Second, social avoidance behavior was developed towards a mouse of the same age and strain. Third, the CSDS induced both short and long-term depression-like behaviors such as social avoidance and behavioral despair; as well as deficits in the long-term memory. In the long-term, the CSDS also induced deficits in learning. Finally, only the chronic administration of CORT (25 µg/ml) induced social avoidance and despair behavior in a similar way that CSDS. In conclusion, the SW strain of mice confers greater validity to CSDS model since the social avoidance behavior was developed towards a mouse of the same strain. Also, mice of the SW strain can be used in the CSDS model as both, aggressors and intruders. Moreover, the CSDS model can induce short and long/term depression-like behaviors and cognitive impairments. Our results underpin the role of CORT in the development of depression-like secondary to chronic stress exposure. Therefore, the CSDS is an animal model useful in the study of the neurobiology of depression and the mechanisms that underlay to the interaction between depression and cognitive impairment.

Disclosures: H.M. Mancha-Gutiérrez: None. **C. Lopez-Rubalcava:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

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Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIMH Grant R01MH114882
NIMH Grant 1R01MH104559-01
NIMH Grant 2R01MH090264-06

Title: Social dominance is associated with resilience and increased active coping behaviors

Authors: *K. B. LECLAIR, K. CHAN, L. F. PARISE, L. LI, F. CATHOMAS, M. P. KASTER, S. J. RUSSO;
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Abstract: The establishment of social hierarchy is an evolutionarily conserved phenomenon that can determine access to resources such as food, water, and potential mates. Across species, rank within social hierarchy can have large effects on health and behavior. Socioeconomic status (SES) has been identified as one of the strongest predictors of mortality and morbidity in humans, with low SES being associated with higher risk of diseases, including cardiovascular disease, cancer, and multiple psychiatric conditions.
Major depressive disorder (MDD) in particular has been associated with low SES even after accounting for lifestyle factors including smoking, alcohol use, physical activity, and diet. However, little is known about the biological underpinnings between the chronic stress of low social status and high disease risk. We seek to investigate this relationship by employing an animal model of chronic stress.
Male C57BL/6J mice in established social hierarchies were exposed to 10 days of chronic social defeat stress (CSDS), composed of a 10 minute encounter with a novel CD-1 aggressor followed by 24 hours of sensory exposure. Behavioral analysis of coping behaviors were assessed across the 10 days of defeat. To investigate the biology underlying the stress response, testosterone and cortisol levels will be evaluated before and after hierarchy formation and defeat. In addition, cFOS activity following the final bout of defeat will be investigated to identify possible neural circuitry involved in the stress response.
Dominant animals show a significantly decreased rate of susceptibility to social defeat stress compared to all other ranks of animals. Dominant animals also show increased active coping behaviors during defeat compared to the most subordinate animals. Taken together these results demonstrate that higher rank in social hierarchy is associated with active coping behaviors during defeat and resilience to CSDS.

Disclosures: **K.B. LeClair:** None. **K. Chan:** None. **L.F. Parise:** None. **L. Li:** None. **F. Cathomas:** None. **M.P. Kaster:** None. **S.J. Russo:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.10/V19

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIMH Grant 1R01MH104559-01
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NIMH Grant 2R01MH090264-06

Title: Chronic social defeat stress increases intestinal permeability and endotoxemia in mice

Authors: *K. L. CHAN, K. B. LECLAIR, F. CATHOMAS, Y. SHIMO, M. P. KASTER, G. PRICE, S. J. RUSSO;
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Abstract: Major depressive disorder (MDD) represents the leading cause of disability, affecting over 300 million people worldwide. Largely characterized by behavioral symptoms, there is a need to identify biological changes associated with MDD. Emerging literature recognizes a correlation between MDD and indices of inflammation, such as increased circulating leukocytes and cytokines. However, it is not fully known how this inflammation is initiated. Recently, several chronic inflammatory conditions have been associated with increased intestinal permeability or 'leaky gut'. We hypothesize that chronic stress compromises the gut barrier, leading to translocation of gut microbial byproducts into circulation, triggering the inflammation associated with depression-like behavior.

To model depression-like behavior in animals, a 10-day chronic social defeat stress (CSDS) model was used, where C57BL/6J mice were exposed to an aggressive CD-1 mouse for 10 minutes per day for 10 consecutive days. Following this 10 minute session, experimental and aggressor mice were co-housed on opposite sides of a divider permitting sensory but not physical contact. Following CSDS, mice were separated into 'susceptible' or 'resilient' groups based on whether they exhibit social avoidance or interaction, respectively. These mice were compared with control mice, which were never exposed to aggressor mice.

To test intestinal permeability following CSDS, mice were orally gavaged with FITC-labelled 4 kDa dextran, with its concentration measured in circulation 1 or 4 hours later. At both time points, blood FITC levels were elevated in mice susceptible to CSDS compared to control or resilient mice, and negatively correlated with individual social interaction ratio. Moreover, circulating bacterial endotoxins, which may arise from gut bacteria, were greater in susceptible mice. To understand molecular mechanisms of stress-induced intestinal permeability, tight

junction expression was evaluated at various regions of the intestine. Several tight junctions, including claudins-5, 8, and 12 were downregulated in the colon from susceptible mice, relative to control or resilient mice. Collectively, these results reveal that CSDS induces breakdown of the intestinal barrier, which may promote systemic inflammation.

Disclosures: K.L. Chan: None. **K.B. LeClair:** None. **F. Cathomas:** None. **Y. Shimo:** None. **M.P. Kaster:** None. **G. Price:** None. **S.J. Russo:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.11/V20

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NC123240.1

Title: Early social isolation effects on amygdala kindling seizures and depressive-like behavior

Authors: *A. VALDÉS-CRUZ, A. E. DÍAZ-FUENTES, C. A. GARCIA-CABALLERO, D. MARTINEZ-VARGAS, V. M. MAGDALENO-MADRIGAL, S. ALMAZÁN-ALVARADO; Lab. de Neurofisiología del Control y la Regulación, Inst. Nacional De Psiquiatría RFM, Cd. De México, Mexico

Abstract: Depression has been widely recognized as a common comorbidity of epilepsy disorders. Factors such as life stress have shown to be more consistent predictors of the development of a comorbid psychiatric condition in epilepsy. The amygdala kindling (AK) model reliably produces seizures of increasing severity with repeated stimulation is a model to assess the pathology progression. Early social isolation (ESI) in rodents is the most well characterized animal model for early stressful experiences and their neurobehavioral consequences, this model could be induced depressive like behavior in adult age. The aim of this study was to analyze the effect of ESI on AK convulsive seizures and depressive-like behavior. At weaning on postnatal day 21, Wistar rats were either group-housed (5 rats/cage), control group (n = 7); or introduced to social isolation by housing them singly, ESI group (n = 7). In adulthood, postnatal day 85-95 rats, a stainless steel tripolar electrode was placed in left temporal lobe amygdala and epidural electrodes in both prefrontal cortices for EEG recording. Daily AK stimulation was done (1 s, 60 Hz, pulse 1 ms, 250-350 μA) until reached three convulsive generalized seizures consecutively. Forced swim test (FST) 5 minutes duration was applied for to evaluate depressive-like behaviors, 24 hours before started AK. Also, focal and generalized seizure susceptibility was applied. Epileptiform spike frequency and after discharge duration in each behavioral AK stages were analyzed. ESI group showed follow results: Increase in epileptic spikes frequency, and decrease in afterdischarge duration of generalized seizures, also an

increase in focal seizure susceptibility was observed. FST test showed greater immobility time, and minor swim time in ESI group. ESI have negative effects in depressive-like behaviors, and on seizures severity, because the pro-depressive process starts in the early stages of development, which could provoked a dysfunction of cerebral structures, as prefrontal cortex and limbic system, both involved into depression-epilepsy comorbidity that is maintained until adult age.

Disclosures: A. Valdés-Cruz: None. **A.E. Díaz-Fuentes:** None. **C.A. Garcia-Caballero:** None. **D. Martinez-Vargas:** None. **V.M. Magdaleno-Madrigal:** None. **S. Almazán-Alvarado:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.12/V21

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIH Grant R21 MH 113899
VA Grant 01 BX000559

Title: Behavioral consequences of inescapable foot shock stress in male rats

Authors: *A. STEPHENS[1], D. A. MORILAK[2,3], D. J. LODGE[2], A. FRAZER[2,3], F. R. CARRENO[2];
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Abstract: Stress is a precipitating factor in the development of both major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). Therefore, we wanted to develop a preclinical model that was sensitive to stress and would produce a variety of behavioral deficits relevant to MDD and PTSD symptoms. An inescapable foot shock stress (IS) was used, in which one session of IS consists of sixty 15 second foot shocks (0.8 mA). Male rats were subjected to two sessions of IS over two consecutive days. The non-shocked (NS) control group was placed in the same chamber for 45 minutes but did not receive any foot shocks. The effect of IS was then evaluated in the female urine sniffing test (FUST) and the shock probe defensive burying test (SPDB). IS caused a significant deficit in the rats' preference for female urine and a shift in coping style in the SPDB, where IS rats spent significantly more time immobile and significantly less time burying the shock probe compared to NS rats. An additional group of male rats underwent IS or NS and were evaluated in the attentional set-shifting task (AST) in order to assess cognitive performance. IS animals had a significant deficit in the first reversal phase and the extra-dimensional set shifting phase of the AST. Additionally, we tested the effect of the α5-$GABA_A$ negative allosteric modulator (NAM), L-655,708 on the behavioral deficits in the

FUST. L-655,708 (3.0mg/kg, I.P.) partially restored the IS-induced reduction of time spent sniffing female urine. Treatment with vehicle, 0.32mg/kg or 1.0mg/kg of L-655,708 had no effect on female urine sniffing. In conclusion, two days of foot shock stress induces hedonic and cognitive deficits relevant for the study of MDD and PTSD.

Disclosures: A. Stephens: None. **D.A. Morilak:** None. **D.J. Lodge:** None. **A. Frazer:** None. **F.R. Carreno:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.13/V22

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: A mouse model of chronic stress-induced social anhedonia: Reversal by leptin

Authors: *Y. LEI, D. WANG, M. GUO, X.-Y. LU;
Dept. of Neurosci. & Regenerative Med., Med. Col. of Georgia at Augusta Univ., Augusta, GA

Abstract: Social anhedonia, a disinterest in social contact and a lack of pleasure in social interactions, is a major symptom of depression. The onset of depression typically occurs during adolescence, a period particularly sensitive to stress, in which social relationships and status are especially salient. Adolescent mice spend more time in social interactions than younger and older animals. We developed a social reward-conditioned place preference (CPP) paradigm in both male and female adolescent mice, which is established via association between the context and rewarding effects of social interaction. Mice at 6 weeks of age are subjected to an initial test (pre-conditioning trial) to establish baseline preference for the two sets of bedding cues, followed by being assigned to receive social conditioning or isolation with two bedding cues. Both male and female mice exhibited a place preference for the socially conditioned context, which was eliminated by pre-exposure to chronic unpredictable stress (CUS). We found that leptin injections enhanced social reward-CPP in control mice and reversed CUS-induced social anhedonia. The mechanisms underlying leptin actions on social reward-CPP are currently being investigated.

Disclosures: Y. Lei: None. **D. Wang:** None. **M. Guo:** None. **X. Lu:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.14/V23

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: GSR Grant

Title: Methionine promotes resilience to chronic social defeat stress by modulating NMDA receptor expression in the cortex

Authors: *M. A. KHALIFE[1], M. BILEN[1], P. IBRAHIM[1], N. BARMO[1], E. ABOU HAIDAR[1], V. JABRE[1], N. KARNIB[1], L. EL HAYEK[1], P. NASRALLAH[1], J. STEPHAN[2], S. SLEIMAN[1]; [1]Natural Sci., [2]Sch. of Med., Lebanese American Univ., Byblos, Lebanon

Abstract: Recent work has focused on epigenetic dysregulation as a major feature of many psychiatric diseases, such as depression. Indeed, individuals affected by depression have disrupted DNA and histone methylation patterns in different regions of their brains. Since methionine (Met), an essential amino acid derived from our diet, is the precursor of S-adenosylmethionine (SAM), a universal methyl donor and cofactor for most methyltransferases, the aim of the study was to examine the effect of Met supplementation on chronic social defeat stress (CSDS), a validated animal model of depression. Male C57BL/6 mice were subjected to chronic social defeat stress for 10 consecutive days, followed by behavioral testing to assess social behavior and anxiety-like symptoms. We found that mice that were subjected to CSDS were susceptible to stress and displayed social avoidance behavior. In contrast, Met treatment promoted resilience to stress as well as rescued social avoidance behavior. Real-time RTPCR analysis of the cortices of these mice revealed that CSDS increased the mRNA expression levels of the NMDA receptor subunits, *Grin1*, *Grin2a* and *Grin2b* and that Met treatment restored their expression levels to baseline. Indeed, by using NMDA receptor activators and inhibitors, we found that Met rescues social behavioral deficits associated with chronic stress by modulating NMDA receptor activity. Finally, we identified the epigenetic mechanism by which Met restored baseline cortical NMDA receptor subunit expression. Indeed, western blot analysis reveals that CSDS decreases the levels of the histone methyl transferase SETDB and its target repressive histone H3 lysine 9 trimethylation (H3Kme3) and Met treatment increases their levels. In conclusion, our work reveals that Met rescues the social behavioral deficits associated with chronic stress by epigenetically regulating the expression of the NMDA receptor subunits.

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Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

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Program #/Poster #: 076.15/V24

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: R01 MH052619
MH065702

Title: Sex-dependent effects of adolescent social isolation on depressive behavior in heterozygous serotonin transporter knockout rats

Authors: *J. L. LUKKES[1], H. L. KLINE[2], A. R. ABREU[1], A. SHEKHAR[3];
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Abstract: Clinical data suggests that carriers of the short allele of the serotonin transporter linked polymorphic region (5-HTTLPR) are at increased risk to develop depression, especially in those individuals exposed to adverse stimuli during critical periods of development. Short allele neurochemical properties of the 5-HTTLPR can be recapitulated in heterozygous serotonin transporter knockout ($SERT^{+/-}$) rats. For instance, a similar reduction in SERT expression is observed in both humans with the 5-HTTLPR short allele and $SERT^{+/-}$ rats. In this study, we aimed to investigate the effects of adolescent social stress in both male and female $SERT^{+/-}$ rats compared to controls on depressive behavior and to determine whether we observe similar behavioral responses in $SERT^{+/-}$ rats as human carriers of the 5-HTTLPR short allele. Male and female $SERT^{+/+}$ and $SERT^{+/-}$ Wistar rats were weaned on postnatal day (P) 21 and then either group- (3/cage) or individually- (1/cage) housed for three weeks from P21-P42. On P39 and P40, rats were assessed in the marble-burying test and for social play behavior. On P42, animals were re-housed in groups of three. Starting on P56, anhedonia was measured using the sucrose preference test. Depressive behavior in the forced swim test was assessed on P72 and animals were perfused 90 min later. Adolescent social stress induced abnormal social play regardless of genotype and sex. Isolation-rearing increased anhedonia in male $SERT^{+/+}$ and $SERT^{+/-}$ rats during adulthood. However, only female $SERT^{+/-}$ rats that were isolated during adolescence displayed an increase in anhedonia. Both group-reared $SERT^{+/-}$ male and female rats exhibited increased immobility, decreased climbing, and decreased swimming in the forced swim test. Adolescent social stress increased depressive behavior regardless of sex or genotype. These data suggest that increased levels of serotonin throughout development increase risk for depressive behavior later in life. Furthermore, our data suggest gene x environment interaction that was observed only in females that increased vulnerability to anhedonia later in life.

Disclosures: J.L. Lukkes: None. **H.L. Kline:** None. **A.R. Abreu:** None. **A. Shekhar:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIH GM109098
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WVU School of Medicine
WVU Research Office

Title: Assessment of mechanisms underlying the impact of the B lymphocyte in the response to stress

Authors: *E. B. E. ENGLER-CHIURAZZI, D. R. CORBIN, J. M. POVROZNIK, B. A. WHITE, A. GUNTER, C. JEBBIA, E. COOK, L. GROSSMAN, J. W. SIMPKINS;
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Abstract: The immune system has emerged as a key regulator of mood, providing novel opportunities for the treatment of debilitating mental health disorders such as major depressive disorder (MDD). Indeed, converging data implicate excessive proinflammatory cascades and a pathological hyper-activation of T cells in the development and persistence of MDD. Yet, the impact of B lymphocytes on the response to stress has not been well studied, leaving a fundamental gap in our knowledge underlying the immune theory of depression. Several emerging findings highlight key roles for B cells in modulating mood. For instance, recently, B cell subtype-specific changes were noted in circulation of MDD patients such that while levels of total B cells were not significantly altered, there were reduced levels of immune-regulating B cells. As well, our discovery of an age-dependent maladaptive response to the acute swim stress in B cell deficient mice and our ability to partially ameliorate this phenotype by replacing B cells, further supports this notion. To expand upon our initial observations, we first administered a B cell depleting antibody (anti-CD20 mAb, 18B12, IgG2a, Biogen, 250µg/mouse) to adult wild type (C57BL6J) mice and evaluated the impact of induced acute B cell deficiency on depressive-like behavior. In a separate study using male B6.129S2-Ighm^{tm1Cgn}/J homozygous mice (aka muMT$^{-/-}$ or BKO) that lack mature B cells and have no membrane-bound IgM expression, we administered purified mouse serum containing immunoglobulins (Sigma Aldrich) to dissociate the observed antidepressant effects of B cell transfer of cells from secretory products produced by the cells. Depressive-like behavior was assayed using the forced swim and/or sucrose preference tests in collaboration with the WVU Rodent Behavior Core facility. Finally, we addressed the age-dependency of our initial observation by harvesting splenic B cells from wild type mice of various ages and conducting a B cell subtype profile evaluation using flow

cytometry in collaboration with the WVU Single Cell and Flow Cytometry Core facility. Initial results indicate that induced acute B cell deficiency does not impact depressive-like forced swim coping behavior nor locomotor or cognitive performance. These data clarify our previous observations regarding B cell deficiency in the context of the response to acute stress. Future directions will address the accumulation of B cells in the brain following a stressor as well as the impact of serotonin stimulation on B cell secreted cytokines.

Disclosures: E.B.E. Engler-Chiurazzi: None. **D.R. Corbin:** None. **J.M. Povroznik:** None. **B.A. White:** None. **A. Gunter:** None. **C. Jebbia:** None. **E. Cook:** None. **L. Grossman:** None. **J.W. Simpkins:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

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Title: The protective effects of Ghrelin/GHSR on hippocampal neurogenesis in CUMS mice

Authors: *H. HUANG, X. CHEN, Q. HAN, J. WANG, Q. LIU, B. LI, G. WU, Y. WANG, J. YU;
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Abstract: Ghrelin is an orexigenic hormone that also plays an important role in mood disorders. Our previous studies demonstrated that ghrelin administration could protect against depression-like behaviors of chronic unpredictable mild stress (CUMS) in rodents. However, the mechanism related to the effect of ghrelin on CUMS mice has yet to be revealed. This article shows that ghrelin (5 nmol/kg, i.p.) decreased depression-like behaviors induced by CUMS and increased hippocampal integrity measured via Ki67, BrdU, doublecortin labeling and Golgi-cox staining, which were decreased under CUMS. Growth hormone secretagogue receptor (Ghsr)-null mice exhibited depression-like behaviors under no stress condition. CUMS induced similar depression- and anxiety-like behavioral manifestations in both Ghsr-null and WT mice. A similar pattern of behavioral changes was observed after hippocampal GHSR knockdown. Additionally,

both Ghsr knockout as well as CUMS exhibited deleterious effects on neurogenesis and spine density in the dentate gyrus. Besides, CCK8 and EdU incorporation assay showed that ghrelin has a proliferative effect on primary cultured hippocampal neural stem cells and this proliferation was blocked by D-Lys3-GHRP-6 (the antagonist of GHSR, 100 μM) pretreatment. Ghrelin-induced proliferation is associated with the inhibition of G1 arrest, and this inhibition was blocked by LY294002 (specific inhibitor of PI3K, 20 μM). Furthermore, the *in vivo* data displayed that LY294002 (50 nmol, i.c.v.) can significantly block the antidepressant-like action of exogenous ghrelin treatment. All these results suggest that ghrelin/GHSR signaling maintains the integrity of hippocampus and has an inherent neuroprotective effect whether facing stress or not.

Disclosures: H. Huang: None. **X. Chen:** None. **Q. Han:** None. **J. Wang:** None. **Q. Liu:** None. **B. Li:** None. **G. Wu:** None. **Y. Wang:** None. **J. Yu:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.18/V27

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Davee Foundation

Title: Immediate acute stress differentially affects interaction with and recognition of inanimate and animate subjects in a genetic rat model of depression

Authors: *A. K. SCHAACK, M. MOCCHI, E. E. REDEI;
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Abstract: In the presence of a familiar and a novel non-threatening animal or object, rats' natural tendency is to explore the novel animal or object. Acute stress is known to interrupt this novelty preference and replace it with a familiarity preference. However, it is not known if stress-reactivity plays a role in this phenomenon and whether there is a difference in stress-induced changes between social and object recognition or novelty preference, respectively. Association with stress-reactivity was studied in a genetic rat model of depression that also shows increased stress-reactivity. The Wistar-Kyoto more immobile (WMI) and less immobile (WLI) rat sub-strains were selectively bred based on their immobility behavior in the forced swim test. The WMI animals consistently show increased stress reactivity compared to the WLI isogenic control, and thus, represent a genetically stress-susceptible rodent model. Adult males and females of both strains were exposed to a 30-minute restraint stress and immediately following stress, exposed to a non-stressful social behavior paradigm using 25-26 days old pups or a novel object recognition (NOR) paradigm. Social interactions of WLI males and WMI

females with a young rat were significantly reduced by acute stress. Acute stress decreased social recognition of the familiar pup in WLI males and WMI females, but increased it in WMI males. Additionally, WMI males, but not females of either strain and WLI males, showed significantly increased aggression after acute stress. In the NOR test, males of both strains investigated the novel object more, with no effect of stress on this novelty preference. In contrast, WLI females increased their investigation of the familiar object after stress showing decreased recognition memory, while WMI females exhibited the opposite pattern. Thus, acute stress affects social interaction and recognition memory differently from that of object recognition, and both sex and stress-reactivity influence these interactions.

Disclosures: A.K. Schaack: None. **M. Mocchi:** None. **E.E. Redei:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

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Program #/Poster #: 076.19/V28

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Davee Foundation

Title: Stress during adolescence sex dependently alters adult affective behaviors in a genetic rat model of depression

Authors: *S. KIM, S. A. GACEK, M. M. MOCCHI, E. E. REDEI;
Dept. of Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: The effect of stress during adolescence is assumed to be detrimental to behavior and cognitive function in adulthood. However, how genetic predisposition to stress-reactivity and depression-like behavior interacts with this developmental challenge is less explored. Therefore, we investigated the effect of stress during adolescence on social, anxiety- and depression-like behaviors and social memory in adulthood using a genetic rat model of enhanced stress-reactivity and depression-like behavior, the Wistar Kyoto More Immobile (WMI) rat strain. The inbred WMI and the isogenic control strain, the Wistar Kyoto Less Immobile (WLI), were generated by bidirectional selection from the Wistar Kyoto using immobility in the forced swim test as a functional selector. Both strains of animals were exposed to contextual fear conditioning (CFC) during adolescence, between 32-34 post-natal days (PND), and were tested after 70 PNDs, as adults. Social interaction with, and recognition of young prepubertal conspecifics, activity and anxiety-like behaviors in the Open Field Test (OFT), and depressive-like behavior in the Forced Swim Test (FST) were assessed. Both WLI and WMI males showed significantly increased social memory and social interaction and decreased anxiety-like behaviors in the OFT after experiencing adolescent stress. However, it increased depressive-like behaviors only in the

WLI males to the levels that of WMI males. In contrast, adolescent stress had no effects on social and anxiety-like behavior in females, but increased depression-like behavior of WLI females to higher levels than that of WMI females. This data is in agreement with findings in human studies that stress during adolescence can be detrimental to some individuals, while others are not affected by adolescent trauma despite exhibiting various risk factors. These behavioral results also question the assumption that stress during adolescence universally leads to adverse behaviors in adulthood.

Disclosures: S. Kim: None. **S.A. Gacek:** None. **M.M. Mocchi:** None. **E.E. Redei:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.20/V29

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Davee Foundation

Title: Stress enhanced fear learning is modified by the intensity of fear conditioning in a genetic rat model of depression

Authors: *K. J. PRZYBYL, S. T. JENZ, P. H. LIM, S. L. WERT, W. LUO, E. E. REDEI; Dept. of Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Posttraumatic stress disorder (PTSD) develops after a major trauma in some individuals, and it is very often co-morbid with major depressive disorder. The Stress-Enhanced Fear Learning (SEFL) model of PTSD was developed to study the mechanism by which stress exposure prior to experiencing a traumatic event increases fear memory in animals, similarly to increasing the likelihood of developing PTSD in humans. It has been shown that the first stressor needs to be of high intensity to generate increased fear memory in the SEFL rat model. However, would fear memory change by exposing the rat to the same first stressor, but altering the intensity of the fear conditioning? Would that change depend on sex and the stress-reactivity of the animal? The genetic model of heightened depression-like behavior and stress-reactivity, the Wistar Kyoto (WKY) More Immobile (WMI), and its isogenic WKY Less Immobile (WLI) control strain were employed. Rats in the prior stress group were restrained for two-hours, 48 hours before contextual fear conditioning (CFC). WLI and WMI male and female rats received three 1 sec foot-shocks in 1 min intervals of either 0.6 mA or 0.8 mA intensity in the conditioning phase of CFC. The rat's freezing responses were considered immediately after the shocks and then 24 h later at the test phase in the same context without shock. WLI males showed no effect of SEFL, either immediately after the shock or at the test phase. Enhanced immediate freezing response and fear memory was observed in the WMI males exposed to prior

stress, but only after 0.8mA intensity CFC. These data suggest that heightened depression-like behavior and stress-responsiveness are risk factors for SEFL in males, while females show a different threshold for fear conditioning intensity and strain-specific coping mechanisms.

Disclosures: K.J. Przybyl: None. **S.T. Jenz:** None. **P.H. Lim:** None. **S.L. Wert:** None. **W. Luo:** None. **E.E. Redei:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.21/V30

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: American Heart Association #19PRE34370044
Scholars in BioMedical Sciences Program (AGK)

Title: Chronic stress induces microglial-mediated inflammatory responses and compromises the oligodendroglial homeostasis during depression

Authors: *A. G. KOKKOSIS, K. VALAIS, M. MULLAHY, S. E. TSIRKA;
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Abstract: Background Major depressive disorder (MDD) is a chronic debilitating illness affecting yearly 350 million people worldwide. Although the mechanisms underlying depression are still not defined, it has been suggested that inflammation acts as depression mediator as it promotes glial and neuronal malfunctions. We have recently established an important association between oligodendrocyte progenitor cells (NG_2-glia) and depression in an adult mouse model of chronic stress (repeated social defeat stress, RSDS). The aim of the pres ent study is to characterize the microglial responses during chronic stress and determine their effects on the NG_2-glial homeostasis and depression onset. **Methods** The RSDS paradigm (10 days) was utilized to study the early (5 days; D5) and post RSDS stages [(10+2 days (D12) and 10+15 days (D25)] in 8-16 week old male C57BL/6J, *CX3CR1*-GFP^+, *CSPG4*-$EGFP^+$ mice. Throughout RSDS, groups were administered BrdU (5-bromo-2'-deoxyuridine) *ad libitum* to monitor cell proliferation. Social interaction (SI) and other behavioral tests (BH) were performed to categorize the defeated mice to susceptible (S) and resilient (R) animals. The study focuses on the MDD-affected Prefrontal Cortex (PFC; n=4-6/per group). Cell quantification and data analysis were blinded and performed by 2 investigators. **Results** Inflammatory responses were observed in the PFC area at D12, depicted by microglial recruitment (Iba1+ cells) and activation [reactive morphology and inflammatory markers (TSPO, CD206, CD86, iNOS, Arg-1)] in the S groups. A corresponding significant decrease of NG_2-glial density (using PDGFRα marker and *CSPG4*-$EGFP^+$ mice) and dramatic morphological alterations were observed in the PFC in S

groups at D12. Given the highly proliferative profiles of NG_2-glia in adult CNS, the mitotic capacity (BrdU, Ki67) was also examined during chronic stress, revealing time-dependent alterations in BrdU labeling retention and proliferation capacity in the S groups. In addition, increased NG_2-glial differentiation was observed (O4+, GST-pi+) in the early RSDS stages of S groups, and a concomitant switch towards NG_2-glial production in the post RSDS stages. Remarkably, after microglial ablation (using the PLX5622 chow diet, Csf1R inhibitor) from D0-D12, mice exhibited resilience to chronic stress as depicted by behavioral and cellular markers. **Conclusions** Chronic stress induces microglial activation and recruitment, swiftly compromising the oligodendroglial homeostasis (proliferation, progeny, morphology), leading to the onset of depressive-like behavior in mice.

Disclosures: **A.G. Kokkosis:** None. **K. Valais:** None. **M. Mullahy:** None. **S.E. Tsirka:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.22/V31

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIMH Grant R01MH082802
NIMH Grant R01MH101980

Title: A rodent model of early life stress (ELS) associated with altered miRNA expression in rat hypothalamus

Authors: *L. A. ALLEN, Y. DWIVEDI;
Psychiatry and Behavioral Neurobio., The Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Early life stress (ELS) is a major risk factor for many psychiatric disorders including major depression (MDD). Nearly 1,000,000 cases of child maltreatment occur in the US each year. It is estimated the MDD affects 15% of Americans, costing $100 billion annually. ELS and MDD are both linked to an increased risk of suicide. Changes in the hypothalamic pituitary adrenal (HPA) axis over the course of development may underlie depression susceptibility after ELS. Although our understanding of the etiology of MDD has substantially improved over the last thirty years, a clear cause and, thus, an effective therapy remain obscure. miRNAs are an epigenetic modifier that can alter gene function post-transcriptionally. Recent studies suggest that miRNAs may play a critical role in MDD pathogenesis, however, only limited research has directly implicated role of miRNA in both ELS and MDD. This study examines how miRNAs are involved in ELS leading to depression in later life.
Male (n=34) and female (n=31) Sprague Dawley pups were maternally separated (MS) for 3 hours per day for the first two weeks of life, postnatal days (PND) 1-14. This protocol was used

to model parental neglect, which has been reported as one of the most common early stressors. As adults (PND 90), the animals were tested for depressive behavior phenotypes and then sacrificed. miRNA expression in the hypothalamus was tested using qPCR. A 2 x 2 ANOVA was used to test for main and interaction effects with follow-up pair-wise T-tests.
We found significant, sex-dependent changes in hypothalamic miRNA expression in a rodent model of ELS. Male MS animals exhibited decreased escape behavior in the forced swim test. On the other hand, both males and female MS animals consumed less sucrose in the sucrose preference test. Among various miRNAs tested, MS increased miR-29 expression in both males and females while miR-124 was increased only in males.
miR-124 and -29 have been previously reported in the MDD and in other ELS rodent models. These miRNAs may underlie the maladaptive changes in HPA function after the experience of ELS. Further elucidating the role of miRNAs in MDD susceptibility after ELS will be key to developing more effective treatments.

Disclosures: L.A. Allen: None. **Y. Dwivedi:** None.

Poster

076. Stress and Mood Disorders: Animal Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 076.23/V32

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: DA039895
MH111604
Bridge of PhD to Neuroscience Program

Title: Characterization of proinflammatory markers in the ventral tegmental area following different forms of chronic stress

Authors: *V. BALI[1], M. RODRIGUEZ[2], M. DOYLE[1], C. MANNING[1], A. ROBISON[1], M. MAZEI-ROBISON[1];
[1]Michigan State Univ., East Lansing, MI; [2]Univ. of Texas at El Paso, El Paso, TX

Abstract: Major depressive disorder (MDD) is one of the most common psychiatric diseases but our understanding of the biological underpinnings remains limited. Rodent models suggest that changes in activity and output of dopamine (DA) neurons in the ventral tegmental area (VTA) are important for depressive-like phenotypes. Additionally, brain inflammatory processes are thought to contribute to MDD pathology and inflammation in the VTA has been shown to alter the activity of VTA DA neurons. Thus, we sought to determine whether there is increased inflammatory signaling in the VTA following forms of chronic stress that induced depressive-like symptoms. First, we subjected male mice to either physical or vicarious chronic social defeat

stress, paradigms known to induce long-term depressive-like behavior and changes in VTA signaling. Second, we subjected male and female mice to subchronic variable stress (SCVS), a paradigm that only induces depressive-like behavior in female mice. We then isolated mRNA from the VTA and assessed proinflammatory gene regulation via RT-PCR. We observed that physical, but not vicarious, CSDS increases interleukin 1β (*Il1β)* mRNA expression and this inversely correlates with social interaction score. In contrast, expression of tumor necrosis factor alpha (*TNFα*) and interleukin 6 were not altered following either form of CSDS. However, *TNFα* expression was decreased in both male and female mice following SCVS, while *Il1β* expression was unchanged. We are now examining additional inflammatory signaling pathways such as toll-like receptor 2 and Cx3 chemokine axis that are involved in microglia/neuronal crosstalk. For example, we find that Cx3cl1 expression is decreased in females, but not males, following SCVS. We hypothesized that VTA microglia, resident macrophages of the brain, are key players in stress-induced neuroinflammatory processes. Therefore, we also performed preliminary analysis of microglia in the VTA via Iba1 immunohistochemistry following stress but we have not assessed morphological markers of activation. Given that the increase in Il1β expression occurred in mice exposed to physical CSDS that also showed the most profound social avoidance, future studies will determine the role of VTA inflammatory signaling in CSDS-induced changes in behavior and VTA DA signaling. In conclusion, we showed that chronic stressors differentially alter expression of proinflammatory genes in the VTA and help to improve our understanding of cellular changes in preclinical models of MDD.

Disclosures: V. Bali: None. **M. Doyle:** None. **C. Manning:** None. **A. Robison:** None. **M. Mazei-Robison:** None. **M. Rodriguez:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.01/V33

Topic: H.01. Animal Cognition and Behavior

Support: NIAA-1R01AA025652-01
NIAA-1P50AA022534-01
NIAA-T32AA014127

Title: Alterations in NMDAR subunit expression and functional behavioral deficits after prenatal alcohol exposure

Authors: *V. LICHERI[1], J. CHANDRASEKARAN[1], J. A. KENTON, JR[1], K. MARQUARDT[1], C. F. VALENZUELA[1,2], J. L. BRIGMAN[1,2];
[1]Dept. of Neurosci., Univ. of New Mexico Sch. of Med., Albuquerque, NM; [2]New Mexico Alcohol Res. Center, UNM Hlth. Sci. Ctr., Albuquerque, NM

Abstract: There is growing evidence that moderate exposure to alcohol during development can lead to behavioral and cognitive deficits that persist throughout the lifespan. Cognitive impairments associated with Fetal Alcohol Spectrum Disorders (FASD) include abnormalities in learning and memory, executive control and social behaviors and are often characterized by a hyper-focus on one particular task or aspect of a task, to the detriment of other important behaviors. We have recently shown that moderate prenatal alcohol exposure (PAE) can impair executive control in adulthood and that behavioral impairments are accompanied by significant alterations in coherence in the orbitofrontal cortex (OFC) and dorsal striatum (dS). Similar deficits and cortico-striatal alterations are found when GluN2B subunit containing N-Methyl-D-Aspartate receptors (NMDAR) are knocked-down in the cortex, and preliminary data shows that GluN2B subunit is significantly reduced in the OFC after PAE. Moreover, recent studies suggest that the GluN2A/GluN2B ratio may act as a "gate" to control induction of synaptic plasticity to support learning or efficient action. Here, we investigated whether behavioral and functional cortical alterations seen after PAE mediated by changes in NMDAR expression and function by integrating a well-established voluntary drinking paradigms for moderate PAE with touch-screen behavioral assays, *in vivo* and *ex vivo* electrophysiology and optogenetic stimulation to potential rescue PAE-induced deficits. First, we examined whether the GluN2A/GluN2B ratio was dynamically altered by training C57BL/6J mice to different stages of discrimination and reversal learning. Next, we performed whole cell recordings from OFC pyramidal neurons in slices obtained from separate cohort of PAE and control mice. NMDA receptor-mediated evoked excitatory post-synaptic currents (NMDA-eEPSCs) were acquired at +40 mV and in the presence of the AMPAR antagonist NBQX (10 µM). GluN2A and GluN2B currents were isolated pharmacologically using PEAQX (1 µM) and Ro25-6981 (1 µM). At the end, the NMDA receptor component was verified applying NMDAR-antagonist (APV 50 µM). Our data show that NMDAR subunit levels are dynamically regulated within the OFC during distinct behavioral stages. Further, PAE alters the function of GluN2B containing NMDAR at the age where behavioral impairments are seen in offspring. Taken together, these findings help elucidate the mechanisms of cognitive impairment in FASD and provide an important tool for developing more effective therapies for executive dysfunction.

Disclosures: V. Licheri: None. **J. Chandrasekaran:** None. **J.A. Kenton:** None. **K. Marquardt:** None. **C.F. Valenzuela:** None. **J.L. Brigman:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.02/V34

Topic: H.01. Animal Cognition and Behavior

Support: 1UH2-MH109168-01

1P50AA022534-01

Title: Cognitive control on the rodent touch-screen five-choice continuous performance task is impaired by prenatal alcohol exposure in a sex dependent manner

Authors: *S. L. OLGUIN[1], D. J. GREGG[1], C. F. VALENZUELA[1], J. CAVANAGH[2], J. L. BRIGMAN[1];
[1]Neurosci., [2]Psychology, Univ. of New Mexico, Albuquerque, NM

Abstract: A common feature of persons with Fetal Alcohol Spectrum Disorder is the inability to concentrate on a specific task while ignoring distractions. Human Continuous Performance Tasks (CPT), typically measure vigilance and cognitive control simultaneously while these are often measured separately in rodents. We have established a touch-screen CPT task that is sensitive to dopaminergic manipulation in mice. Here, we examined the effects of a moderate prenatal alcohol exposure (PAE) on CPT performance in adult male and female mice obtained from the NMARC where PAE is established via a drinking-in-the-dark model (10% sweetened EtOH available 4 hours/day, throughout gestation; BAC: ~90mg/dL; controls=0.066% sweetened water, "SAC"). In early-adulthood, mice were trained on the 5-Choice CPT task (5C-CPT). Briefly, mice were first trained to touch a single stimulus in 1 of 5 available windows ("target" trial) once responding quickly and accurately "non-target" trials were added in which all 5 squares were illuminated and mice only receive reward for withholding responding. Results showed that all mice were able to perform the single stimuli stage equally regardless of sex or treatment. With the addition of hold trials, PAE mice made significantly increased false alarms (responses to non-target stimuli) on hold trials versus controls. Compared to SAC, PAE mice had a significantly lower d prime, indicating they responded more often to non-target stimuli than target stimuli. Female mice, regardless of treatment, completed fewer overall trials, had fewer premature responses, and omitted more target trials. Next, we examined the effects of PAE on neurophysiological activity during the 5C-CPT via dura-resting EEG-like recording. PAE and SAC animals were trained to respond to stimuli and then fitted with EEG caps targeting medial prefrontal, parietal and motor cortices with a ground over the cerebellum. Dura-resting EEG-like recording was conducted during the 5:1 ratio of the CPT. Results suggest that PAE animals had decreased in theta (4-10Hz) oscillatory signaling in the medial prefrontal cortex during target trials. Similarly to non-exposed C57BL/6J, parietal beta (13-25Hz) power also was altered during non-target trials. These results utilizing dura-resting EEG-like recording suggests that alterations in cortical activity after withholding a response may underlie deficits in cognitive control after PAE. Together, these findings suggest that moderate exposure to alcohol during development can have long lasting effects on attention, vigilance, and inhibition.

Disclosures: S.L. Olguin: None. **D.J. Gregg:** None. **C.F. Valenzuela:** None. **J. Cavanagh:** None. **J.L. Brigman:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.03/V35

Topic: H.01. Animal Cognition and Behavior

Support: 1R01AA025652-01
1P50AA022534-01
T32AA014127

Title: Optogenetic rescue of cortico-striatal dysregulation and behavioral flexibility deficits following prenatal alcohol exposure

Authors: *J. CHANDRASEKARAN, J. KENTON, V. LICHERI, C. F. VALENZUELA, J. L. BRIGMAN;
Univ. of New Mexico, Albuquerque, NM

Abstract: Growing evidence demonstrates the negative effects moderate alcohol consumption during pregnancy has on executive function. Moderate prenatal alcohol exposure (PAE) via a drinking in the dark paradigm (10% EtOH available 4 hours/day throughout gestation; BAC: ~90mg/dL; SAC: saccharine controls) impairs executive function by significantly increasing perseveration on a touchscreen-based visual reversal learning task. Single unit and local field potential (LFP) activity during behavior was significantly altered in the orbitofrontal cortex (OFC) during early reversal. We also observed decreases in synchronous activity between the OFC and dorsal striatum (dS). These findings suggest that deficits in behavioral flexibility after PAE occur due to impaired neuronal functioning/coordination during reversal learning. Here, we investigated if alterations in inhibitory/excitatory signaling contribute to behavioral deficits seen after PAE. First, to investigate alterations in GABAergic tone, behaviorally naïve, food restricted PAE/SAC mice were sacrificed at 8-10 weeks old. Using IHC, perfused brains(50μm) were labeled for PV^+, SST^+ and CR^+ interneurons and were counted using unbiased stereology. A separate cohort was trained, then microinfused with channelrhodopsin-expressing adeno-associated virus (AAV-CAMKαII-ChR2(H134R)-mCherry), fitted with recording optrodes targeting the OFC/dS for recording/stimulation. After 4 weeks, PAE/SAC mice re-attained discrimination criteria and were tested on reversal. On sessions 1-4 of reversal, PAE/SAC mice received light pulses (10 Hz, 5mW, 5ms pulse for 1s) or no stimulation, 1 second following a correct choice while recording waveform/LFP activity. To test if behavioral deficits can be rescued via modulation of OFC-dS projections specifically, we used retrograde expression of ChR2 in the dS to tag OFC neurons andtest if stimulation of these neuronswas sufficient to restore efficient reversal learning. Unbiased stereology indicates an increase in the number of SST^+/ CR^+populations of interneurons in the OFC of PAE mice vs SAC controls, but no changes

in the PV^+population. Direct stimulation of OFC increased firing *in vivo* and coordinated activity following correct choices and reduced perseveration in PAE mice compared to controls. These data provide evidence that PAE may alter excitatory/inhibitory balance at baseline and stimulation of specific populations of cortical neurons may reduce the impaired flexibility seen after PAE. Current studies are examining the role of direct OFC-dS projection via retrograde labelling combined with *ex vivo* electrophysiology in PAE/SAC control animals.

Disclosures: J. Chandrasekaran: None. **J. Kenton:** None. **V. Licheri:** None. **C.F. Valenzuela:** None. **J.L. Brigman:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.04/V36

Topic: G.08. Drugs of Abuse and Addiction

Title: Are there common neural substrates of cocaine craving and post-cocaine working memory deficits in rats?

Authors: *M. SCHWENDT, C. M. GOBIN;
Psychology, Univ. of Florida, Gainesville, FL

Abstract: Cocaine use disorder (CUD) is associated with prefrontal cortex dysfunction and cognitive deficits that may contribute to persistent relapse susceptibility. While it has been proposed that interventions addressing both cognitive and motivational deficits might provide better treatment outcomes, possible neural substrates that play a dual role in post-cocaine cognitive deficits and cocaine craving remain poorly understood. We have recently developed an animal model that allows for an investigation of 'incubated' cocaine craving and working memory capacity in rats. Using this model, we have recently described that rats with a history of extended access cocaine self-administration display demand-dependent working memory deficits that are related to prior cocaine intake and later drug-seeking. We have also found that in cocaine rats hypermetabolic activity within the prelimbic cortex is related to working memory performance and that in the same brain region cocaine-associated cues elicit a robust expression of immediate early gene *Arc*. Our next goal was to investigate a possible overlap in patterns of neural activity evoked by performing working memory task and/or induced during cocaine-seeking. In the initial study, adult male rats were trained to respond in an operant delayed match-to-sample (DMS) working memory task. Next, rats' performance in the DMS task were tested across a range of delays (0-24s). As expected, demand-dependent decrease in working memory performance occurred as delays (working memory load) increase. After characterizing baseline working memory capacity, a single 30-minute DMS test was conducted in which rats were tested under high-demand (24s delay), low demand (0s delay) conditions, or remained in their home

cage. Again, rats tested under high-demand working memory conditions displayed significantly poorer performance when compared to their low-demand counterparts. Analysis of the rat brain tissue collected immediately after the test revealed demand-dependent increase in *c-fos* mRNA expression in the prelimbic cortex. Furthermore, the majority of *fos*-positive cells were also positive for metabotropic glutamate receptor 5 mRNA, suggesting activation of pyramidal neurons. Our follow-up studies are aimed to investigate 1) a possible overlap between the population of cells activated during high-demand working memory and during relapse to cocaine-seeking using mRNA mapping and genetic tools; and 2) the effects of cell-type specific inactivation within the prelimbic cortex on both working memory and cocaine-seeking.

Disclosures: M. Schwendt: None. **C.M. Gobin:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.05/V37

Topic: G.08. Drugs of Abuse and Addiction

Title: Interaction of mGlu5 and A2a receptors in regulation of working memory and cocaine-seeking

Authors: *P. U. HAMOR, C. M. GOBIN, M. SCHWENDT;
Psychology, Univ. of Florida, Gainesville, FL

Abstract: Cocaine use disorder (CUD) is characterized by compulsive drug-seeking and persistent relapse vulnerability. Chronic cocaine use also produces a range of cognitive deficits, with attention, working memory (WM), and impulsivity being the most affected. Prefrontal cortex (PFC) hypofunction has been found to coincide with these deficits. The hypofunction seems to correlate with the length of cocaine use and decreased working memory performance. Further, the magnitude of post-cocaine cognitive deficits also predicts increased likelihood of relapse. However, the relationship between post-cocaine WM deficits and cocaine-seeking, or the identity of possible shared neurobiological substrates have not been thoroughly investigated in animal models. We have previously found mild but persistent WM deficits in rats with a history of extended access to cocaine when compared to controls. Here we investigated cocaine-induced changes in WM performance in adult male rats. We used a within-subject design for delayed match-to-sample task. Delays renged 0-24 seconds. We found significant overall decrease of WM score in rats with history of cocaine self-administration (6 days of 1 hr/day, 12 days of 6 hr/day access to cocaine) when compared to their pre-cocaine baseline. Because inhibition of mGlu5 receptors with negative allosteric modulators (NAMs), such as MTEP, reliably reduces cocaine relapse in animals, these compounds have been proposed as a potential treatment for CUD. However, its negative effects on cognition have been previously reported.

On the other hand, antagonism of adenosine 2a receptor (A2a), a receptor which interacts with mGlu5, has been shown to increase WM performance in both naïve and impaired animals. Therefore, we proposed a combined pharmacological treatment A2a receptor antagonist KW-6002 (istradefylline) and MTEP. We hypothesized that this combined treatment might decrease the rate of relapse to cocaine-seeking due to the inhibition of mGlu5 receptors, while KW-6002 would counteract the negative effects of MTEP on WM performance. Here we show that both chronic cocaine and systemic administration of MTEP (1 mg/kg) significantly impair WM. However, the WM performance was not improved or rescued by KW-6002 (0.125, 1 mg/kg) pretreatment. Moreover, combination of MTEP and KW-6002, while having no effect on WM, significantly increased relapse to cocaine-seeking. These results are in support of cocaine- and MTEP -induced WM impairment that is insensitive to A2a receptor manipulations. Future studies will investigate possible changes in mGlu5 and A2a interactions in relation to post-cocaine motivational and cognitive impairments.

Disclosures: P.U. Hamor: None. **C.M. Gobin:** None. **M. Schwendt:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.06/V38

Topic: H.01. Animal Cognition and Behavior

Support: NIDA Grant 5R21DA043722-02

Title: Cannabis vapor self-administration during adolescence abolishes sex differences in cognitive flexibility in adulthood

Authors: *T. G. FREELS[1], H. R. WRIGHT[1], N. C. GLODOSKY[2], A. M. HAMPTON[3], X. L. HERRERA[3], M. W. MELVILLE[3], R. J. MCLAUGHLIN[1];
[1]Integrative Physiol. and Neurosci., [2]Dept. of Psychology, [3]Washington State Univ., Pullman, WA

Abstract: As more states accommodate for the legalization of cannabis, the social stigma and perceived harms associated with cannabis use continue to decline. Cannabis use during sensitive developmental periods, particularly during adolescence, has become a major public health concern because its long-term effects on cognitive function remain largely unknown. Given that adolescence is a critical period for the development of brain regions implicated in higher-order cognitive functioning, this study sought to investigate possible behavioral deficits in cognitive flexibility in adult rats that volitionally consumed cannabis during adolescence. Male and female Sprague-Dawley rats (n=12-14/sex/group) were trained to self-administer vaporized cannabis extracts high in delta-9-tetrahydrocannabinol (THC_E), cannabidiol (CBD_E), or a vehicle solution

containing 80% propylene glycol and 20% vegetable glycerol from postnatal (PND) day 35-55. On PND 65, rats began training in an automated attentional set-shifting task designed to assess alterations in behavioral flexibility. An effort discounting task was also utilized to evaluate the effects of adolescent cannabis use on effort-based decision making in adulthood. Results indicate that female rats self-administered more THC_E vapor (but not CBD_E vapor) than males during adolescence. In the attentional set-shifting task, notable sex differences were observed such that females performed better when shifting to an egocentric spatial cue. Additionally, adolescent THC_E vapor self-administration abolished this observed sex difference, impairing set-shifting and increasing regressive errors committed by female rats. Interestingly, there was no correlation between the number of vapor deliveries received during the maintenance phase of self-administration and the number of regressive errors made during the reversal learning component of the task in females. This suggests that the effect on set-shifting performance is not solely due to differences in cannabis self-administration. There was no effect of adolescent cannabis self-administration on effort-based decision making in either sex. Collectively, these data indicate that female rats self-administer more THC_E vapor during adolescence, and that adolescent females exposed to THC_E vapor may be at greater risk for developing cognitive deficits in adulthood. Our findings also support the validity of the cannabis vapor self-administration approach to further interrogate the effects of cannabis use on behavioral and cognitive outcomes.

Disclosures: T.G. Freels: None. **H.R. Wright:** None. **N.C. Glodosky:** None. **A.M. Hampton:** None. **X.L. Herrera:** None. **M.W. Melville:** None. **R.J. McLaughlin:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.07/V39

Topic: H.01. Animal Cognition and Behavior

Support: MH081843
MH098631

Title: Psychostimulants exert dose dependent effects on frontostriatal neuronal signaling

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Abstract: The prefrontal cortex (PFC) and extended frontostriatal circuitry play a critical role in higher cognitive function. Dysregulation of frontostriatal-dependent cognition is implicated in a variety of behavioral pathologies, including addiction and ADHD. Psychostimulants exert potent

dose-dependent cognitive actions. Specifically, at higher doses associated with psychostimulant abuse, these drugs robustly impair frontostriatal-dependent cognition. In contrast, low doses used in the treatment of ADHD, improve PFC-dependent cognitive function. Currently, our understanding of the neural coding bases for the diverse cognitive actions of psychostimulants are unclear. To address this, we examined the effects of cognition-impairing and cognition-enhancing doses of methylphenidate (MPH) on neuronal spiking activity and local field potential (LFP) power spectral density within the dorsomedial PFC (dmPFC) and dorsomedial striatal (dmSTR) in rats performing a spatial working memory task. This circuit has been shown previously to support working memory. Within the dmPFC, a *cognition-impairing* dose of MPH robustly suppressed the activity of neurons strongly tuned to delay and reward, while activating neurons not tuned to a tone signaling a correct choice prior to reward delivery. In contrast, in the dmSTR, cognition impairing doses of MPH had no effect on neurons strongly tuned to task events, while robustly increasing firing of neurons not strongly tuned to these events. Thus, cognition-impairing doses strongly decrease the signal-to-noise representation of key task events. Interestingly, *cognition improving* doses had minimal impact on task-related firing of neurons in either region. For LFP spectral density, MPH elicited a complex array of actions that were region-, frequency- and dose-dependent. Interestingly, in the PFC, we saw opposing actions of cognition improving vs. cognition impairing doses of MPH on the ratio of theta power to both beta and gamma power. Specifically, cognition enhancing doses increased while cognition impairing doses suppressed these ratios. These ratios have been previously linked to both cognitive function and PFC dysregulation. Lastly, delay-related PFC-STR coherence in the gamma range was increased selectively by the cognition-impairing dose of MPH. These observations indicate that the cognition-improving vs. cognition-impairing effects of psychostimulants target unique aspects of frontostriatal neuronal coding.

Disclosures: R.C. Spencer: None. **A.J. Martin:** None. **D.M. Devilbiss:** None. **R.L. Jenison:** None. **C.W. Berridge:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.08/V40

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH116526
NIH Grant MH081843
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Title: Stress disrupts cognition-related neuronal coding within frontostriatal circuitry

Authors: *A. MARTIN[1], D. M. DEVILBISS[2], R. C. SPENCER[1], R. L. JENISON[1], C. W. BERRIDGE[1];
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Abstract: Acute and chronic stress is well-documented to impair higher cognitive function dependent on the prefrontal cortex (PFC) and extended frontostriatal circuitry. Stress-related deficits in frontostriatal cognitive function are observed in multiple psychopathologies as well as the majority of work-place accidents. Currently, our understanding of the neural mechanisms underlying the cognition-impairing actions of stress is limited. To address this knowledge gap, we utilized ensemble recordings to determine the effects of acute noise stress on neuronal spiking and local field potential (LFP) activity within dorsomedial frontostriatal circuitry in rats tested in a delayed-response task of working memory. For this, 8 wire electrode arrays were chronically implanted in the dorsomedial PFC and dorsomedial striatum (STR). This circuit has been demonstrated to play a pivotal role in higher cognitive function, including working memory. Waveform analyses identified individual wide-spiking, putative projection neurons in the PFC and medium spiny neurons in the STR across 40-trial baseline and 40-trial stress exposure or no-stress (control) recording sessions. Within the PFC, stress robustly degraded the population size and spiking activity of wide-spiking, putative projection neurons strongly tuned to the delay interval and reward. In contrast, stress increased spiking activity of neurons that were weakly tuned to delay. This latter observation indicates, stress is not simply increasing the inhibitory tone within the PFC. Within the STR, stress elicited a weaker suppression of delay-related firing rate and population size while having no impact on reward-related signaling. Oscillatory activity contained within LFPs help regulate/coordinate neuronal signaling within and between regions. Of particular relevance, theta activity in the PFC is positively correlated with working memory. We observed that stress-related cognitive impairment was associated with a robust suppression of theta and alpha power in both the PFC and STR as well as theta- and alpha-coherence between these regions. In contrast, stress increased beta related coherence. These observations indicate that stress disrupts multiple components of frontostriatal neuronal signaling that supports higher cognitive function.

Disclosures: A. Martin: None. **D.M. Devilbiss:** None. **R.C. Spencer:** None. **R.L. Jenison:** None. **C.W. Berridge:** None.

Poster

077. Cognitive Effects of Abused Substances

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.09/V41

Topic: H.01. Animal Cognition and Behavior

Support: NIDA Grant DA014339

Title: A history of cocaine increases impulsivity and disrupts neural activity in the prelimbic cortex

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Abstract: Individuals with a history of cocaine use have heightened impulsivity. However, little research has investigated the changes in neural activity associated with this increase. To assess this, we examined neural activity in the prelimbic cortex (PrL) and nucleus accumbens (NAc) core, two brain regions implicated in impulsivity, during a behavioral inhibition task in animals following a brief abstinence from cocaine self-administration. In this task, rats (n=20) were trained to wait for the presentation of a light cue before pressing a lever to receive a sucrose pellet. Presses on the lever that occurred before cue presentation were considered 'impulsive' and were not rewarded. After training, we recorded electrophysiological activity (single-unit and local field potential, LFP) during the task. Subsequently, animals underwent two weeks of self-administration for either water/saline or cocaine (1 mg/kg/inf) for 6 hr/day. Finally, on days 4-6 of abstinence from self-administration, we reassessed impulsivity and electrophysiological activity. We found that animals with a history of cocaine had significantly heightened impulsivity compared to control rats. Additionally, during the task these animals had altered neural activity in the PrL, but not NAc core. Specifically, immediately before pressing the lever, naïve and water/saline rats exhibited a brief inhibition in mean PrL neural activity. This brief pause in firing was abolished or even reversed in rats with a history of cocaine. Additionally, rats with a history of cocaine had heightened PrL 10 Hz oscillatory activity immediately following extension of the lever into the chamber. Furthermore, each rat's shift in PrL neural activity (single-unit or oscillatory LFP) during the task significantly correlated with that rat's shift in impulsivity. In conclusion, our findings suggest that the PrL, but not the NAc core, tracks heightened impulsivity in animals with a history of cocaine self-administration.

Disclosures: T.M. Moschak: None. **R.M. Carelli:** None.

Poster

077. Cognitive Effects of Abused Substances

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 DA034021
NIH Grant T32 DA007244

Title: Behavioral effects of prior cocaine exposure on unsignaled delay-based decision making and prelimbic cortical activity

Authors: *M. L. NGBOKOLI, T. M. MOSCHAK, R. M. CARELLI;
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Abstract: Individuals with a substance use disorder often have heightened impulsivity, including an inability to delay gratification (known as delay discounting). Delay discounting relies upon the ability to discriminate both different delays to reward as well as different reward magnitudes, and work has begun to investigate the distinct effects of drugs of abuse on delay and magnitude sensitivity. Our lab recently found that a history of cocaine self-administration impairs magnitude discrimination and abolishes dopamine encoding of food reward in the nucleus accumbens (NAc) shell (Saddoris et al., Neuropsychopharm, 2017). However, little research has investigated the neurocircuitry underlying cocaine's effects on delay-based decision making. Although the NAc shell is important in magnitude processing, the NAc core seems to play a stronger role in delay processing. Additionally, the prelimbic cortex (PrL), which projects to the NAc core, exhibits neuroadaptations following cocaine abstinence (West et al., Eur J Neurosci, 2014) and is implicated in delay discounting (Churchwell et al., Behav Neurosci, 2009). Here, we investigated the effects of cocaine self-administration history and abstinence on delay-based decision making and PrL activity using electrophysiological methods. Adult, male Sprague Dawley rats (n=7) were trained to self-administer cocaine (0.33 mg/inf, 2 h per session) or water with yoked intravenous saline (n=9 rats) during 14 daily sessions, followed by 30 days of experimenter-imposed abstinence. Rats were then trained to press levers to receive a reward in a delay task consisting of three trial types. On forced delay trials, a lever extension predicted the opportunity for a delayed reward (4 s delay). On forced immediate trials, another lever predicted the opportunity for an immediate reward. During free choice trials, both levers were presented, and rats could freely choose either option. Data collected indicate that rats with a history of cocaine chose the delay lever less during free choice trials ($t = 2.637$, $p = 0.0120$). Preliminary results also suggest a shift in neural population activity during free choice trials; animals with a history of cocaine had significantly fewer cells phasically respond to the lever when they ultimately chose the delayed reward ($p = 0.0128$) and more excitatory cells when they ultimately chose the immediate reward ($p = 0.0369$) when compared to control animals. In total, these data suggest that a history of cocaine biases behavior and PrL activity in favor of the immediate reward.
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Disclosures: **M.L. Ngbokoli:** None. **T.M. Moschak:** None. **R.M. Carelli:** None.

Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

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Program #/Poster #: 077.11/V43

Topic: H.01. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation
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Title: Breakdown of stable delay discounting behavior following methamphetamine administration

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Abstract: Delay discounting, or the degree to which rewards are devalued by delays preceding their arrival, is a critical factor in a variety of life outcomes and is altered in numerous psychiatric conditions. In particular, individuals suffering from substance use disorders display enhanced discounting of delayed rewards, with a concomitant increase in preference for immediately-available rewards (i.e., immediate gratification). For individuals suffering from methamphetamine addiction, the relapse rate is alarmingly high; one study found that 61% of methamphetamine users relapse within their first year of recovery (Brecht et al., 2014). It has been shown that methamphetamine can alter dopaminergic activity (Lin et al., 2016) and can lead to deficits in memory for up to 21 days (North et al., 2013). Given the many deleterious effects of methamphetamine on brain function, it is feasible that it could also alter perception of rewards and their associated costs. To determine how methamphetamine affects delay discounting, rats were tested in a spatial delay discounting task before, during, and after exposure to the drug. When performing the task, rats were prompted to choose between a small food reward with a 1-second delay, and a larger food reward at an initial cost of a 30-second delay. The delay for the larger reward was dynamic, however, in that the delay increased after each large reward delivery and decreased after each small reward delivery. The “indifference point”, identified by averaging the delays in the last twenty trials in each session, was used as an index of rats’ preferred delay for a larger reward (Papale et al., 2012). Rats were trained until reaching a stable indifference point (2.9% variance across 4 days). Following a single injection of

methamphetamine (2.0 mg/kg, ip), however, the variance in indifference points increased (8.6% variance across the 4 days post-injection). Similar effects were observed following 5 days of daily methamphetamine injections (2.0 mg/kg), in that variance increased to 11.8%. Notably, variance in indifference points remained elevated above baseline (6.8%) one month after repeated methamphetamine administration. These results show that even relatively brief exposure to methamphetamine can have lasting effects on decision making behavior, which may have implications for understanding its effects in the context of substance use disorders.

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Poster

077. Cognitive Effects of Abused Substances

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH085739
NIH Grant GM113109

Title: Effects of methylphenidate on impulsive choice and timing processes in Lewis rats

Authors: *K. PANFIL, R. SMALL, K. KIRKPATRICK;
Kansas State Univ., Manhattan, KS

Abstract: Attention-Deficit/Hyperactivity Disorder (ADHD), a common behavioral disorder in children and young adults, is characterized by symptoms of impulsivity, inattention, hyperactivity, and poor temporal perception. The Lewis rat, a proposed model of ADHD, was compared to its control strain, the Wistar rat, on a series of tasks on and off methylphenidate (MPH)—a commonly prescribed medication for ADHD. Rats were trained to ingest MPH orally via syringe and were then tested across a range of doses (0-10 mg/kg). Impulsivity was measured with an impulsive choice task where rats chose between a smaller-sooner (SS) reward or a larger-later (LL) reward. Off MPH, Lewis rats made fewer LL choices than Wistar rats. Analyses of acquisition suggest that Lewis and Wistar rats learned the impulsive choice task at a similar rate, but the Lewis rats chose the LL less often when the delay to reward was the same as the SS option. This may be due to a developed aversion to the LL lever, which was associated with the longest delays to reward. Higher doses of MPH improved Lewis rats' impulsive choice behavior but worsened Wistars' behavior. Temporal perception (i.e., accuracy and precision) was evaluated with a peak-interval task on a 30-s interval. Off MPH, Lewis rats had poorer temporal precision compared to Wistar rats but similar accuracy in timing the delay. Interestingly, Lewis rats had higher response rates as well, suggesting hyperactivity. Higher doses of MPH shifted

Lewis rats' accuracy closer to the target interval while Wistars shifted away from the interval. All doses of MPH improved temporal precision across strains. In sum, Lewis rats may be a viable model for both ADHD and delay aversion, which is the avoidance of long delays. This strain exhibits symptoms of impulsivity and poor temporal precision, but further investigation is needed for assessing symptoms of inattention and hyperactivity. Lewis rats may be a promising model for testing time-based interventions to improve impulsive choice through attenuation of delay aversion.

Disclosures: K. Panfil: None. **R. Small:** None. **K. Kirkpatrick:** None.

Poster

077. Cognitive Effects of Abused Substances

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 AA17986-01A1
NIH Grant R01 AA25289-01
NIH Grant S10OD018132

Title: Chronic alcohol induced liver injury correlates with memory deficits and neuroinflammation

Authors: *J. A. KING[1], B. NEPHEW[2], G. POIRIER[3], A. CHOUDHURY[3], A. LIM[3], P. MANDREKAR[3];
[2]Biol. and Biotech., [1]Worcester Polytechnic Inst., Worcester, MA; [3]Psychiatry, Ctr. for Comparative NeuroImaging, Umass Med. Sch., Worcester, MA

Abstract: Alcohol use disorder (AUD) affects over 15 million adults over age 18 in the United States, with estimated costs of 220 billion dollars annually—mainly due to poor quality of life and lost productivity, which in turn is intricately linked to cognitive dysfunction. AUD induced neuroinflammation in the brain, notably the hippocampus (hippocampus), is likely to contribute to cognitive impairments. The neuroinflammatory mechanisms mediating the impact of chronic alcohol on the central nervous system, specifically cognition, require further study. We hypothesized that chronic alcohol consumption, impairs memory and increases inflammatory cytokines TNFα, IL6, MCP1, and IL1β in the hippocampus and prefrontal cortex (prefrontal cortex) regions in the brain. Using the chronic-binge Gao-NIAAA alcohol mouse model of liver disease, representative of the drinking pattern common to human alcoholics, we investigated behavioral and neuroinflammatory parameters. Our data show that chronic alcohol intake elevated peripheral and brain alcohol levels, induced serum alanine aminotransferase (ALT), a marker of liver injury, impaired memory and sensorimotor coordination, increased inflammatory

gene expression in the hippocampus and prefrontal cortex. Interestingly serum ALT and hippocampal IL-6 correlated with memory impairment, suggesting an intrinsic relationship between neuroinflammation, cognitive decline, and liver disease. Overall, our results point to a likely liver-brain functional partnership and suggest that future strategies to alleviate hepatic and/or neuroinflammatory impacts of chronic AUD may result in improved cognitive outcomes.

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Poster

077. Cognitive Effects of Abused Substances

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 077.14/V46

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R21 DA040559

Title: Using fMRI to further understand sexual HIV-risk decision-making in stimulant users and controls

Authors: *T. D. WILLSON[1], J. LISINSKI[2], M. W. JOHNSON[3], S. M. LACONTE[2], H. U. DESHPANDE[2], W. K. BICKEL[2], *M. N. KOFFARNUS[2];
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Abstract: Individuals with stimulant use disorder have a higher rate of HIV infection due to an increase in sexual HIV-risk behavior. In previous studies, discounting of delayed monetary rewards and delayed safer sexual encounters was greater in adults with stimulant use disorder than in controls. Studies using functional magnetic resonance imaging (fMRI) showed recruitment of the frontal-parietal-limbic valuation network during monetary discounting tasks by measuring blood oxygen level dependent (BOLD) responses, but neural recruitment during intertemporal risky sex choices is not known. Here, we assessed neural correlates of sexual-risk decision making between men and women with stimulant use disorder and controls. This exploratory analysis focused on differences in BOLD responses between those who self-reported risky sexual behavior and those who did not, independent of stimulant use status. Sixty-one subjects participated in monetary and sexual delay discounting while undergoing fMRI. Stimulant users were selected using DSM-V criteria. Risky sexual behavior was assessed using the risk assessment battery and HIV risk-taking behavior scale. Discounting rates and brain activity via BOLD responses were compared. During monetary discounting choice trials, those who reported risky sexual behavior showed increased BOLD responses of the thalamus, cingulate gyrus, and prefrontal cortex. During sexual delay discounting, risky sexual behavior

interacted with delay revealing increased responses in the insula and precuneus. Additionally, when viewing images of hypothetical sexual partners identified as most versus least attractive, participants who reported risky sexual behavior had increased responses in the precuneus and lingual gyri. Increased activity within the thalamus suggests increased communication between the frontal lobe and basal ganglia in the risky sex participants. While BOLD responses were higher in the prefrontal cortex, the actual functional ability in these regions appears diminished for this group. The differential activity in the visual cortices of those with risky sex behavior may be due to perception of future motor movement (i.e. sexual activity). Finally, the activation of the insula could be suggestive of risk assessment during intertemporal choice.

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Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.01/W1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA026820

Title: Gpr88 agonist decreases excessive alcohol intake in mice

Authors: S. BEN HAMIDA[1], E. CLARKE[1], E. MORONCINI[1], E. DARCQ[1], *C. JIN[2], B. L. KIEFFER[1];
[1]Douglas Mental Hlth. Inst. and Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada; [2]RTI Intl., RTP, NC

Abstract: The current therapies for alcohol abuse disorders have little effectiveness and continued development of pharmacotherapies is needed. One target that has generated recent interest is the neural orphan G protein-coupled receptor GPR88. Total deletion of *Gpr88* gene in mice leads to a range of phenotypes including motor coordination deficits, as well as altered cue-based and procedural learning, which is consistent with the strong striatal Gpr88 expression. Interestingly, we found an enhanced motivation to seek and consume alcohol in Gpr88 knockout animals, associated to reward hypofunction and reduced neural communication between the prefrontal cortex and nucleus accumbens in these animals[1]. Importantly, the first potent, selective, and brain-penetrant GPR88 agonist (RTI-13951-33) was recently discovered[2]. RTI-13951-33 exhibited an EC50 of 25 nM using an *in vitro* cAMP functional assay and had no significant off-target activity in a selectivity panel with more than 50 GPCRs, ion channels, and neurotransmitter transporters. As behavioral validation, RTI-13951-33 significantly reduced alcohol operant self-administration without effects on locomotion and sucrose self-

administration in rats. Here, we further investigate the effect of RTI-13951-33 on excessive alcohol intake in mice, in order to confirm the action of RTI-13951-33 in another species and test specificity of the agonist in Gpr88 knockout mice. Specifically, we evaluated the effect of RTI-13951-33 using a 20% alcohol intermittent-access two-bottle-choice drinking procedure. First, adult male Gpr88 knockout mice and controls were exposed to alcohol to develop preference and divided into two groups of equal alcohol consumption, and then treated with single intraperitoneal injection of saline or RTI-13951-33 (30 mg/kg). Thirty minutes later alcohol and water intake were assessed for 24 hrs. Control animals treated with RTI-13951-33 showed reduced alcohol intake and preference. No significant effects on alcohol consumption or preference were observed in Gpr88 null mutant mice. This result concurs with the previous rats results, and demonstrates the specificity of RTI-13951-33 in vivo, at least for the alcohol response. Our data therefore put forward the possibility that targeting GPR88 is an innovative and valuable potential strategy for the treatment of alcohol use and abuse disorders. 1. Ben Hamida, S. *et al. Biol Psychiatry* **84**, 202-212, doi:10.1016/j.biopsych.2018.01.026 (2018). 2. Jin, C. *et al. J Med Chem* **61**, 6748-6758, doi:10.1021/acs.jmedchem.8b00566 (2018).

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Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.02/W2

Topic: G.08. Drugs of Abuse and Addiction

Title: Lowering of dietary n-6 polyunsaturated fatty acid lowered voluntary ethanol binge drinking in mice

Authors: *C. T. CHEN[1], S. HAVEN[1], K. SCHUEBEL[2], D. GOLDMAN[3], J. R. HIBBELN[4]; [1]NIH, Rockville, MD; [2]Johns Hopkin Univ., Baltimore, MD; [3]Lab. Neurogenetics, Natl. Inst. on Alcohol Abuse and Alcoholism Lab. of Neurogenetics, Rockville, MD; [4]LMBB, NIH/NIAAA, Rockville, MD

Abstract: Research Objectives and Rationale: Previously, a study had shown that different oil source may alter ethanol preference in hamsters; however, it is uncertain if this translate into ethanol consumptions. Therefore, our aims are two-fold: 1) to examine the effects of lowering dietary omega-6 polyunsaturated fatty acids (n-6 PUFA, linoleic acid; LA) and/or supplementation of omega-3 polyunsaturated fatty acids (n-3 PUFA, eicosapentaenoic acid and docosahexaenoic acid; EPA and DHA) on voluntary ethanol binge drinking and 2) to examine the pathway networks involved in the interactions of polyunsaturated fatty acids and ethanol in striatum. **Methods:** Behavioral cohort: Time-pregnant C57BL/6J dams were randomized to one

of four custom dietary interventions varied in the combination of n-6 PUFA (8 energy% linoleic acid or 1 en% linoleic acid) and n-3 PUFA (0.5 en% EPA+DHA or 0 en% EPA+DHA). The fatty acid compositions of diets are crafted based on contemporary American and evolutionary model human intake. Male offspring continued their respective maternal diet for 8 weeks before drinking-in-the-dark paradigm (n=16-18). RNA-Seq cohort: Same dietary interventions were applied. 15-week-old male offspring was subjected to a 9-day 2 g/kg and 1-day 5 g/kg ethanol gavage paradigm. Striatum were collected for next generation RNA sequencing after the last dose of gastric gavage. Sex differences are not explored in this study. **Summary of results:** Mice fed 1 en% LA and 0 en% EPA+DHA (L6L3) lowered voluntary ethanol binge drinking by 29% for the final 4 consecutive weeks as compared to mice fed 8 en% LA and 0 en% EPA+DHA (H6L3). Mice fed n-3 supplements did not differ in ethanol binge drinking as compared to L6L3 and H6L3 mice. Mice fed L6L3 exhibited more differentially expressed genes in striatum as compared to other dietary interventions (L6L3, 158 genes; H6L3, 16; L6H3, 39; H6H3, 20). Pathway analysis showed that striatal transcriptomic signatures in response to ethanol gavage are different between dietary interventions. **Conclusion:** Dietary fatty acids may alter striatal transcriptomic response to ethanol which may result in lowering of voluntary ethanol binge drinking. Dietary lowering of n-6 PUFA may be effective in preventing adolescent/young adult alcohol binge drinking.

Disclosures: C.T. Chen: None. **S. Haven:** None. **K. Schuebel:** None. **D. Goldman:** None. **J.R. Hibbeln:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

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Program #/Poster #: 078.03/W3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
NIH Grant DA035958

Title: Cluster of differentiation 5 knockout mice display reduced ethanol consumption and resistance to ethanol induced sedation

Authors: *J. D. OBRAY, A. J. PAYNE, B. WILLIAMS, J. BALDRIDGE, J. T. YORGASON, K. S. WEBER, S. C. STEFFENSEN;
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Abstract: Cluster of differentiation 5 (CD5) is expressed in both T and B cells. CD5 has been found to display an altered expression profile following chronic ethanol use and during ethanol withdrawal. Specifically, the number of CD5+ B cells is reduced during withdrawal while the

number of T cells is increased. Given the apparent sensitivity of these cells to ethanol and recent research suggesting that some ethanol effects are accounted for by neuroimmune interactions we assessed drinking behavior and ethanol induced sedation in CD5 knockout (KO) mice. We found that CD5 KO mice display decreased ethanol consumption as compared with wild-type controls and that ethanol consumption does not increase with repeated exposure in CD5 KO mice. Additionally, CD5 KO mice displayed considerable resistance to the sedating effects of ethanol. Further studies are underway to assess whether there are baseline differences in dopamine dynamics within the mesolimbic pathway between CD5 KO mice and wild-type controls as well as to whether neurons in the mesolimbic pathway differ in their response to ethanol in CD5 KO mice.

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Poster

078. Mechanisms Underlying Alcohol Consumption I

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Program #/Poster #: 078.04/W4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant P20GM113109
Kansas State University

Title: Voluntary alcohol consumption in adolescent and adult rats: Consumption patterns and effects on taste reactivity

Authors: *T. J. WUKITSCH[1], T. J. MOSER[1], J. GOMENDOZA[1], P. M. SMALL[1], C. CUNNINGHAM[1], J. P. RACK[1], M. ALLISON[1], M. CAIN[2];
[1]Psychlogical Sci., Kansas State Univ., Manhattan, KS; [2]Psychological Sci., Kansas Sate Univ., Manhattan, KS

Abstract: Individuals exposed to alcohol during adolescence are more likely to develop alcohol problems later in life. It is unknown whether alcohol exposure during adolescence changes the hedonic value of alcohol similar to the way exposure in adulthood affects alcohol's hedonic value. Further, it is unclear whether adolescent alcohol exposure affects the hedonic value of other non-drug rewards in adulthood. Therefore, the current study examined the relationship between voluntary alcohol consumption during adolescence or adulthood and taste reactivity to alcohol and sucrose in adulthood. Sixty-four male Long-Evans rats reared in the facility until beginning intermittent access to 20% v/v ethanol (IAE) 3 days/week (2-bottle-choice) on postnatal day (PND) 27 for adolescents and PND 76 for adults. Controls received only water during IAE. After 6-weeks of IAE blood alcohol analysis was performed. Analysis of IAE

indicated that, while adolescents had higher mean drinking overall, they had a significant decline in drinking over time compared to adults that escalated drinking over time. However, the higher mean drinking and decline in drinking appears to be driven by high drinking in adolescents during the first session of IAE. When analyzed excluding only the first IAE session, no differences in drinking between adults and adolescents emerged and model fit improved. Following completion of the IAE phase, rats were implanted with an intraoral fistula and, following recovery, were tested for their taste reactivity to water, alcohol (5, 20, & 40% v/v), and sucrose (0.01, 0.1, 1M). Hedonic and aversive taste reactions to alcohol were affected by IAE and alcohol concentration tested, but not age. Compared to water controls, IAE rats had greater hedonic responses and less aversive responses as concentration of alcohol increased. However, IAE did not affect hedonic or aversive responding to sucrose. Surprisingly, adults had greater hedonic responding to sucrose, however, while both age groups increased hedonic responding as sucrose concentration increased, adolescents had a much steeper increase. These findings suggest that adolescents and adults voluntarily consume alcohol similarly on average except during initial contact with alcohol. Further, changes in hedonic value and aversion to alcohol caused by IAE are similar between adult and adolescent rats. The findings also indicate that IAE, regardless of age, does not alter the hedonic value of sucrose rewards. Taken together, these results suggest that, while IAE increases hedonic and reduces aversive responses to alcohol, the impact does not vary as a function of the age at which IAE occurs.

Disclosures: T.J. Wukitsch: None. **T.J. Moser:** None. **J. Gomendoza:** None. **P.M. Small:** None. **C. Cunningham:** None. **J.P. Rack:** None. **M. Allison:** None. **M. Cain:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.05/W5

Topic: G.08. Drugs of Abuse and Addiction

Title: Gene expression changes associated with anxiety like-behaviors and alcohol escalation

Authors: *E. D. BARBIER, R. BARCHIESI, K. CHANTHONGDEE, M. HEILIG, E. DOMI; Ctr. for Social and Affective Neuroscience, IKE, Linkoping, Sweden

Abstract: Comorbidity of alcohol use and anxiety disorders is a major cause of disability and a challenge for mental health services. Both disorders are characterized by broad and persistent changes in gene expression within brain areas involved in regulation of negative affect including the prefrontal cortex and the amygdala. However, the shared underlying mechanisms are still not well known. In our study, we used a rat model of social defeat stress (SDS) to assess the impact on alcohol- and anxiety-like behaviors. In addition, a second group of rats were made to witness the SDS in order to unravel the psychological component from the combined physical and

psychological stress in the defeated animals. In accordance with previous studies, we found individual variability in the behavioral outcomes following social stress. Stress induced by social defeat or by witnessing SDS, led to an increase in operant alcohol self-administration and anxiety-like behaviors only in a subset of animals. Behavioral studies were performed ten days after the last social defeat, suggesting a long lasting effect of the social stressors on both alcohol intake and anxiety-like behaviors. Gene expression changes observed on the subset of rats showing both alcohol and anxiety-related behaviors are assessed using our custom made NanoString panel. Our panel comprises about 400 genes involved in critical neuronal functions such as neurotransmitter release and synaptic plasticity. It also include epigenetic regulators. This is of particular interest as stress and heavy alcohol have been shown to reprogram the transcriptome, making interventions that target epigenetic mechanisms an attractive novel approach to develop therapeutics.

Disclosures: E.D. Barbier: None. **R. Barchiesi:** None. **K. Chanthongdee:** None. **M. Heilig:** None. **E. Domi:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.06/W6

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant AA022408
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NIH grant T32GM065823
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Title: Synthetic peripherally restricted cannabinoid suppresses voluntary alcohol intake in rats via non-CB1/CB2 receptor action

Authors: *V. N. MARTY[1], Y. MULPURI[1], A. S. DHOPESHWARKAR[2], J. J. MUNIER[1], A. LIAO[1], S. LELE[1], R. H. VO[1], I. YENOKIAN[1], K. MACKIE[2], I. SPIGELMAN[1];
[1]Div. of Oral Biol. & Medicine, Sch. of Dent., Univ. of California Los Angeles, Los Angeles, CA; [2]Psychol & Brain Sci., Indiana Univ. Bloomington Dept. of Psychological and Brain Sci., Bloomington, IN

Abstract: Relapse to alcohol (ethanol, EtOH) is a critical problem in treating alcoholism and the federally-approved medications to treat alcohol use disorders (AUDs) have shown limited

efficacy in terms of craving and relapse rate reduction. We recently demonstrated that both the peripherally-restricted cannabinoid receptor agonist 4-{2-[-(1E)-1[(4-propylnaphthalen-1-yl)methylidene]-1H-inden-3-yl]ethyl}morpholine (PrNMI) and the brain-permeant WIN 55,212-2, decrease chronic-pain-induced escalated voluntary EtOH intake and preference in rats. Here we studied the mechanisms by which PrNMI suppresses EtOH intake. Male Wistar rats were placed on an intermittent 2-bottle choice (2BC) 10% (w/v) EtOH regimen. Rats were given access to water and EtOH for a 24-hr period (3 days/week) and only water on remaining days. After achieving stable EtOH intake levels, rats (n=15) were injected either with vehicle (DMSO:Tween80:saline at 1:1:10 ratio) or PrNMI (0.6 mg/kg, i.p.). In another cohort, rats (n=10) were injected either with peripherally restricted CB1R antagonist 18A (3 mg/kg, i.p.), selective CB2R antagonist SR144528 (3 mg/kg, i.p.), or a combination of PrNMI+18A or PrNMI+SR144528. In a different cohort of rats (n=8), blood from the tail vein was collected 1 hr after vehicle or PrNMI injection. PrNMI induced a long-lasting significant decrease in EtOH intake and preference. Selective blockade of peripheral CB1Rs or CB2Rs did not affect EtOH intake and preference. Furthermore, co-administration of PrNMI with 18A or SR144528 did not prevent PrNMI-induced decreases in EtOH intake and preference. In vitro studies showed that PrNMI produced concentration-dependent activation and internalization of GPR119, whose activation in vivo leads to glucagon-like peptide 1 (GLP-1) release. Analysis of plasma [GLP-1] revealed increases after PrNMI versus vehicle injections. Our study shows that PrNMI-induced decreases in voluntary EtOH intake are not mediated by peripheral CBR activation. However, PrNMI effects may involve GPR119-induced increases in GLP-1. These findings provide new insights into the complex mechanisms involved in alcohol intake, and suggest that development of peripherally selective novel drugs may prove useful in treating AUDs.

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Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.07/W7

Topic: G.08. Drugs of Abuse and Addiction

Support: Wu Tsai Neurosciences Institute

Title: Association of mesolimbic responses to alcohol cues with current alcohol intake in human adolescents

Authors: *S. I. HUDSON, K. H. MACNIVEN, B. KNUTSON;
Psychology, Stanford Univ., Stanford, CA

Abstract: After arriving at college, adolescents face numerous inducements to initiate or increase their alcohol consumption. Failure to successfully regulate alcohol intake can have a number of negative consequences, ranging from academic difficulties to increased risk of lifelong substance dependence. In this baseline assessment of a longitudinal study, we examined whether individual differences in mesolimbic responses to alcohol cues might index heightened alcohol consumption in a sample of non-disordered college freshman similarly to patients with alcohol (Reinhard et al., 2015) and stimulant use disorders (MacNiven et al., 2018).
Sixty first-year college students (mean age: 18.33, 29 females) completed an event-related cue-reactivity FMRI task that included images of alcohol, food and neutral items (18 per condition). Following the scan, each participant was interviewed about their alcohol consumption over the past 30 days using the timeline follow-back method, and additionally asked to report on their average alcohol consumption over the past 6 months.
Among the subset of participants who had consumed at least one full drink in their lifetime (n=47), increased ventral tegmental area (VTA) responses to alcohol vs. neutral cues correlated with greater alcohol use in the past 30 days, past 6 months, and overall (past 30 days + past 6 months; $r=.476$, $p<.001$; $r=.457$, $p<.001$; $r=.474$, $p<.001$), as well as greater binge drinking in the past 30 days ($r=.475$, $p<.001$). Increased left Nucleus Accumbens (NAcc) responses to alcohol vs. neutral cues were also correlated with greater alcohol use in the past 6 months and overall ($r=0.34$, $p=.019$, $r=.314$, $p=.032$).
Together, these results suggest that mesolimbic responses to alcohol cues are associated with current drinking behaviors in college students. The findings extend previous literature suggesting that medial prefrontal cortical responses to alcohol advertisements are associated with current drinking behavior in college students (Courtney et al., 2018) to subcortical regions implicated in reward processing (i.e., the VTA and NAcc). Going forward, longitudinally tracking these subjects will allow us to determine whether mesolimbic responses to alcohol cues predicts future increases in drinking. Further, comparison with a clinical sample will illuminate whether these associations hold in patients with an alcohol use disorder.

Disclosures: **S.I. Hudson:** None. **K.H. MacNiven:** None. **B. Knutson:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.08/W8

Topic: G.08. Drugs of Abuse and Addiction

Support: Swedish Medical Research Council Grant
Stiftelsen Psykiatriska Forskningsfonden
Swedish Brain Foundation
Alcohol Research Council of the Swedish Alcohol Retailing Monopoly

Bror Gadelius Minnesfond
Wilhelm och Martina Lundgrens vetenskapsfond
LUA/ALF Grant

Title: Combination treatment with varenicline and bupropion eliminates alcohol deprivation effect in rats

Authors: *K. DANIELSSON[1], A. DE BEJCZY[2], L. ADERMARK[1], M. ERICSON[1], B. SÖDERPALM[1,2];
[1]Addiction Biol. Unit, Dept. of Psychiatry and Neurochemistry, Univ. of Gothenburg, Gothenburg, Sweden; [2]Beroendekliniken, Sahlgrenska Univ. Hosp., Gothenburg, Sweden

Abstract: Alcohol use disorder (AUD) is a major contributor to global disease burden and hence also potentially one of the largest preventable causes of poor health. However, available treatment options only work for a fraction of the patients. Thus new and efficient treatments are highly needed. Nicotine use, primarily in the form of cigarette smoking, and AUD are closely linked with high co-morbidity and pharmacological similarities, as dopamine (DA) release in the nucleus accumbens (nAc) caused by both substances involves activation of nicotinic acetylcholine receptors (nAChRs), albeit of different subtypes. It has also been demonstrated that nicotine exposure can increase alcohol consumption and *vice versa*. It is therefore possible that AUD could be treated using substances efficient in smoking cessation. Indeed, the partial nAChR agonist varenicline was recently shown to reduce alcohol intake in man, an effect possibly involving interference with nAChRs of importance for ethanol-induced DA release as well as enhanced DA levels caused by the partial nAChR activation. This latter effect could possibly be augmented by co-administration of the DA/noradrenaline reuptake inhibitor bupropion. Indeed, varenicline and bupropion show an additive effect on smoking cessation, whereas the combined effect of these agents on alcohol intake has not previously been examined. We therefore investigated the effects of varenicline, bupropion or a combination of the two on nAc DA levels, as well as on ethanol intake and the alcohol deprivation affect (ADE).
Adult male Wistar rats were used for all experiments. *In vivo* microdialysis showed that systemic administration of both varenicline (1.5 mg/kg) and bupropion (2.5, 5 or 10 mg/kg) elevate nAc DA levels. Furthermore, the combination of the two treatments produced an additive effect. Voluntarily ethanol drinking animals were treated for five days with varenicline (1.5 mg/kg), bupropion (2.5 mg/kg) or the combination, and during the time of treatment there was no effect on ethanol consumption when compared to vehicle treated controls. However, following a 14-day abstinence period, when ethanol was reintroduced, varenicline, but not bupropion, tended to reduce the ADE and the combined treatment with varenicline and bupropion completely blocked the ADE.
Due to the fact that both compounds have previously been found safe to use across various populations, and that the ADE has a high predictive value for clinical outcome in humans, the present findings suggest that the combination of varenicline and bupropion could be a promising treatment for AUD.

Disclosures: **B. Söderpalm:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder

(pending) for the combination of substances, Intellectual property rights. **K. Danielsson:** None. **A. De Bejczy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder (pending) for the combination of substances, Intellectual property rights. **L. Adermark:** None. **M. Ericson:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.09/W9

Topic: G.08. Drugs of Abuse and Addiction

Support: Butler University Holcomb Faculty Research Grant

Title: Observing the effects of combined alcohol and caffeine on somatic withdrawal signs in C57BL/6J mice

Authors: M. D. JENKINS, ***J. N. BERRY**;
Psychology, Butler Univ., Indianapolis, IN

Abstract: Caffeine is one of the most widely used psychoactive stimulants in the world and is often used in combination with other substances. The combination of caffeine and alcohol has been shown to induce a stimulated, rather than sedated state, which may result in increased alcohol-attributable accidents (e.g., drunk driving, unprotected sex, and extreme intoxication). Preclinical studies have shown mixed results regarding the co-consumption of caffeine and alcohol - some found that caffeine increases alcohol intake while others the opposite. The current study expanded on previous research by testing the effects of combined caffeine and alcohol exposure in a binge-like, mouse paradigm. It was anticipated that the mice would consume larger amounts of alcohol in combination with caffeine compared to mice consuming either alcohol or caffeine alone. The present study explored drinking behaviors in 24 adult C57BL/6J mice using an intermittent access 2-bottle choice paradigm. Singly-housed mice were presented with one bottle of tap water and one bottle of tap water with incrementally increasing concentrations of alcohol (3-20% v/v), caffeine (0.01-0.05% w/v), or a mixture of alcohol and caffeine every other day. Approximately 24 hours after the last drinking day, mice were videotaped to assess somatic signs of alcohol and/or caffeine withdrawal. We hypothesized that mice in the combined alcohol and caffeine condition would drink more than in the other conditions and that their physical dependence, as evidenced by increased somatic signs, would be greater as opposed to either drug alone. Exposure to combined alcohol and caffeine did not significantly alter consumption or preference for alcohol, but did significantly increase caffeine consumption and preference compared to caffeine alone. Exposure to alcohol, caffeine, or the combination of alcohol and caffeine did not result in significant anxiety-like behavior as measured by somatic signs. The

current study shows that the intermittent access model is a viable tool to study caffeine and alcohol co-consumption. Given the mechanistic overlap between the two substances and the large number of individuals who are co-dependent on both, future studies should continue to examine the effects of combined alcohol and caffeine.

Disclosures: M.D. Jenkins: None. **J.N. Berry:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.10/W10

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA019793

Title: Testing the contribution of social context on preclinical efficacy of potential treatments for alcohol use disorder

Authors: *M. T. ROBINS, A. E. RYABININ;
Dept. of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: To date, only three drugs are available for alcohol use disorder (AUD) treatment, and their use is restricted by inconsistent efficacy and/or high rates of patient noncompliance. The lack of progress in developing new AUD therapeutics may be surprising given the plethora of preclinical research studies identifying novel targets that modulate alcohol behaviors in rodent models. For example, drugs identified preclinically as efficacious in decreasing alcohol intake in rodents, such as corticotropin-releasing factor (CRF) receptor antagonists, have failed to show efficacy in humans. This lack of translation may be the result of absences in essential biological homology between humans and rodents; however, a simpler cause may be differences in social environment during testing, as rodents are commonly tested in isolation despite social pressure being one of leading causes of AUD relapse in humans. Here, we assessed the influence of social environment on baseline alcohol intake and on treatment efficacy in C57Bl/6 male, adult mice housed in either groups of four animals or in isolation using the HM-2 cage system - a novel system that monitors individual animals' water and alcohol intake behaviors using radio frequency identification. In a continuous access, two-bottle choice model (water vs. 4-8% alcohol), we observed no significant differences in alcohol intake between mice housed in isolation versus those housed socially, although mice housed in isolation displayed more variable drinking patterns during the first few days of testing. On the 10^{th} day of drinking, we assessed the ability of a selective CRF1 receptor antagonist, CP-376,395, to decrease alcohol intake at a dose previously identified to decrease alcohol intake in individually-housed mice in standard cages (20 mg/kg). In the HM-2 cages, a decrease in alcohol intake was observed in animals housed

individually; however, no change in alcohol intake was observed in mice housed socially. Our results suggest that social environment may alter the efficacy of drugs known to decrease alcohol intake in rodents housed in isolation, and that considering social environment is vital to potentially increase the translational value of rodent models of AUD.

Disclosures: M.T. Robins: None. **A.E. Ryabinin:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.11/W11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA R37AA01684 (DR)

Title: Bdnf projections from the orbitofrontal cortex to the dorsolateral striatum regulate alcohol drinking behaviors and habit

Authors: *J. J. MOFFAT, S. A. SAKHAI, Y. EHINGER, K. PHAMLUONG, D. RON; Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: We previously showed that brain-derived neurotrophic factor (BDNF) signaling in the dorsolateral striatum (DLS) gates alcohol self-administration in rodents [1]. In this study, we mapped the circuitry underlying BDNF's actions on alcohol intake. First, we discovered that one of the principal brain regions sending BDNF-positive efferents to the DLS is the orbitofrontal cortex (OFC), a brain region integral in decision making and updating the value of predicted outcomes [2]. With this in mind, we hypothesized that BDNF-expressing neurons in the OFC-DLS projection moderate the degree of alcohol intake. Overexpression of BDNF in the OFC, which activated BDNF signaling in the DLS, was sufficient to reduce voluntary consumption of, and preference for, 20% alcohol. In contrast, saccharine intake was unaltered. Furthermore, overexpression of BDNF in the motor cortex did not alter alcohol intake. Importantly, pathway-specific overexpression of BDNF in OFC neurons projecting to the DLS led to an attenuation of alcohol intake and preference. Taken together, these findings indicate a noteworthy role for OFC-DLS BDNF signaling in preventing excessive alcohol-drinking behavior in mice. Next, we set out to determine how BDNF in OFC-DLS circuitry gates alcohol intake. Because the DLS plays an important role in habit formation [3], we hypothesized that BDNF in the OFC to DLS projection gates habitual alcohol seeking. We found that mice trained to habitually seek alcohol demonstrated a shift from habitual to goal-directed alcohol seeking in a single contingency degradation session following systemic treatment with LM22A-4, an agonist for the BDNF receptor TrkB. Conversely, systemic TrkB activation has no effect on mice trained to self-administer alcohol in a goal-directed manner. Thus, BDNF signaling biases habitually-trained

mice toward goal-directed alcohol seeking behavior.
1. Logrip et al., Brain Research, 2015. 2. Wallis, J.D., Annu Rev Neurosci, 2007. 3. Yin and Knowlton, Nat. Rev. Neurosci, 2006.

Disclosures: **J.J. Moffat:** None. **S.A. Sakhai:** None. **Y. Ehinger:** None. **K. Phamluong:** None. **D. Ron:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.12/W12

Topic: G.08. Drugs of Abuse and Addiction

Support: ZIA-AA000421

Title: Mu opioid receptor in direct-pathway medium spiny neurons contribute to the preference for alcohol drinking

Authors: *A. MATSUI[1], V. A. ALVAREZ[2];
[1]NIAAA/NIH, Bethesda, MD; [2]Lab. on Neurobio. of Compulsive Behaviors, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: Endogenous opioid peptides are released in the nucleus accumbens (NAc) following alcohol exposure. The opioid release is thought to be a critical step associating with the rewarding property of alcohol. My goal is to elucidate how alcohol exposure modulate mu-opioid receptor (MOR) function in the NAc and how it contributes to the development of alcohol use disorder.
The two subclasses of neurons, direct- (dMSNs) and indirect-pathway medium spiny neurons (iMSNs), in the NAc express opioid peptides and opioid receptors; however, it is unclear whether MOR is expressed in subclasses of MSNs. Opioid receptors are inhibitory G-protein coupled receptor. One main effect of opioid receptor activation in these MSNs is to depress GABAergic synaptic transmission from these neurons into the target area in the ventral pallidum. Here we evaluated the expression pattern of opioid receptors by assess the degree of inhibition in presynaptic transmitter release using subtype specific opioid agonists. Our results showed that both dMSNs and iMSNs express functional mu-, and kappa-opioid receptors, but not delta-opioid receptors at the presynaptic terminals. Next, we generated a mouse lines with targeted deletion of MORs to dMSNs or iMSNs in order to evaluate the role of MOR in alcohol drinking behavior. The results showed that mice lacking MOR in dMSN have lower preference for alcohol drinking in two-bottle choice paradigm, suggesting a role for MORs specifically on dMSNs in alcohol drinking. However, mice lacking MOR in iMSNs did not reveal the difference

in alcohol drinking behaviors. The results of these studies reveal that MOR activation in dMSN might underlie the behavioral transition to alcohol abuse.

Disclosures: A. Matsui: None. **V.A. Alvarez:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.13/W13

Topic: G.08. Drugs of Abuse and Addiction

Support: Sponsored Research at Ithaca College

Title: Single or repeated mild traumatic brain injury during adolescence does not significantly affect ethanol consumption or cause long-term behavioral alterations in Sprague-Dawley rats

Authors: E. P. GAVRILLES, J. E. MILLER, *T. L. DOREMUS-FITZWATER; Psychology, Ithaca Col., Ithaca, NY

Abstract: Adolescents across a variety of species have been shown to exhibit more risk-taking behaviors—including drinking alcohol—when compared to adults. Given these behaviors, and their involvement with sports, adolescents are also at a higher risk for concussion. Importantly, some research has reported that traumatic brain injury (TBI) may increase alcohol consumption. The current experiment, therefore,sought to explore the relationship between TBI and alcohol intake by investigating whether mild TBI (mTBI) during adolescence might lead to immediate and/or lasting changes in ethanol drinking and other behaviors. To do this, male and female Sprague-Dawley rats (N = 64) began drinking ethanol in early adolescence (postnatal day [P] 30 ± 2) using the intermittent access 2-bottle choice (IA2BC) paradigm. During a 2-week period (7 total drinking sessions) rats were given a choice between water and sweetened ethanol [10% (v/v) with 3.0% sucrose (w/v) and 0.125% saccharin (w/v)] every Monday, Wednesday and Friday for approximately 23 h per day. After two drinking days, rats were assigned to a TBI group: single mTBI, 4 intermittent mTBIs, or sham control. A closed-head weight drop model (150 g at 1 m) was used to induced mTBI. Animals in the mTBI conditioned received a single mTBI and then continued to drink ethanol without further injury. Those experiencing multiple mTBIs, however, received 3 more mTBIs that were interleaved with ethanol drinking days. Sham controls were treated identically but did not receive any blows to the head. Following the 7thdrinking session, animals matured to adulthood for 6 weeks without further ethanol access or injury. The IA2BC procedure was then resumed for an additional 4 weeks to assess any long-term consequences of adolescent mTBI on adult ethanol consumption. A 1-week washout then occurred, followed by a behavioral test battery. Results showed that, during the adolescent period, neither single nor multiple mTBI(s) changed ethanol intake in male or female rats. Once

in adulthood, ethanol consumption decreased compared to adolescence, but was still unaffected by adolescent mTBI. Depression-like behavior and motor behavior in the sucrose preference test and Rotarod, respectively, were also unchanged in mTBI rats compared to sham controls. Together, these results suggest that, in this model of mTBI, few consequences of adolescent head injury are observed for ethanol drinking, affective, or motor behaviors. Current and future experiments are exploring other possible cognitive and psychological behavioral impairments that may be evident after adolescent concussion, as well as alterations in brain function and inflammation.

Disclosures: E.P. Gavrilles: None. **J.E. Miller:** None. **T.L. Doremus-Fitzwater:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.14/W14

Topic: G.08. Drugs of Abuse and Addiction

Support: Alcohol Research Foundation

Title: The lack of conditioned place preference but unaltered stimulatory and ataxic effect of alcohol in the mGluR3-KO mice

Authors: *T. AITTA-AHO, M. LAINIOLA, A.-M. LINDEN, L. HIETALA;
Univ. of Helsinki, Helsinki, Finland

Abstract: Alcohol use associates with environmental cues that can later reinstate drinking patterns without any alcohol exposure. Alcohol-induced reward, when combined with contextual signals of various sensory modalities in the central synapses of mesolimbic reward circuitries, can lead to the formation of conditioned responses. As the activation of glutamatergic synapses is pivotal in such processes, we aimed to investigate whether metabotropic glutamate receptor subtype 3 (mGluR3) plays a role in alcohol-induced behaviors including place preference. An mGluR3 deficient mouse line (mGluR3-KO) was used to study alcohol-induced place preference, locomotor activating and ataxic effects, limited access alcohol drinking, and preference for sucrose and saccharin. Alcohol-induced horizontal locomotor stimulation and reduced rearing behavior remained unchanged in the mGluR3-KO mice. However, alcohol-induced place conditioning in an unbiased paradigm setup was lacking in the mGluR3-KO mice, but clearly present in the WT mice. Locomotor activity was not different between the mGluR3-KO and WT mice during the acquisition and expression trials. Alcohol consumption, studied through the ‘drinking in the dark’ model, remained unchanged in the mGluR3-KO mice. The mGluR3-KO mice also showed normal sucrose and saccharin preference. These studies indicate

a role for mGluR3 in the conditioned contextual alcohol responses, but not in stimulatory, and ataxic alcohol effects.

Disclosures: **T. Aitta-Aho:** None. **M. Lainiola:** None. **A. Linden:** None. **L. Hietala:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

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Program #/Poster #: 078.15/W15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant F31 AA027420-01
U01 AA014095
U24 AA020929
P50 AA010761
T32 AA007474
VA Medical Research (BX000813)

Title: Extended amygdala dynorphin/kappa opioid receptor activity contributes to excessive binge-like ethanol consumption in male and female mice

Authors: *H. L. HAUN[1], T. KASH[2], H. C. BECKER[3];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]UNC- Chapel Hill, Chapel Hill, NC;
[3]Charleston Alcohol Res. Ctr., Med. Univ. South Carolina, Charleston, SC

Abstract: Alcohol Use Disorder is a significant national and global public health problem. Of concern, binge drinking is the most common pattern of excessive alcohol (ethanol) consumption. We have recently implicated the dynorphin/kappa opioid receptor (DYN/KOR) system in a model of rodent binge-like ethanol consumption (Drinking-in-the-Dark paradigm). More specifically, we found that DYN-containing neurons within the central amygdala (CeA) contribute to binge drinking, but a role for the projection targets of these neurons is not known. The bed nucleus of the stria terminalis (BNST) is rich in KOR, receives DYN input from the CeA, and has been implicated in binge drinking. The current studies were designed to probe the role of KOR activity within the BNST and determine the contribution of DYN projections in the CeA-BNST circuit to binge-like ethanol consumption in male and female mice. Briefly, mice were given access to a single 20% ethanol bottle (v/v) for an extended 4 hr limited-access binge session. Microinjection of a KOR antagonist (nor-BNI; 2.5 µg/side) into the BNST robustly reduced binge ethanol drinking in both male (3.9 vs 1.7 g/kg, $p<0.05$) and female mice (4.7 vs 2.2 g/kg, $p<0.05$). Importantly, this dose of nor-BNI did not affect locomotor activity in an open field task. In a separate experiment, systemic administration of a KOR agonist (U50,488; 5 mg/kg) resulted in an increase in ethanol intake compared to vehicle (4.08 vs 5.17 g/kg; $p<0.05$).

Furthermore, the effect of systemic U50,488 was reversed by intra-BNST nor-BNI (5.17 vs 1.70 g/kg; $p<0.05$). Lastly, DYN-containing neurons projecting from the CeA to the BNST were targeted using a chemogenetic approach to selectively 'silence' the CeA-BNST^{DYN+} pathway prior to binge drinking in both male and female Pdyn-IRES-Cre mice. Briefly, AAVrg-hSyn-DIO-hM4Di-mCherry (0.3 µL/side) or control virus were infused into the BSNT and guide cannulae were positioned above CeA to target cell bodies in the CeA-BNST^{DYN+} circuit with clozapine-*N*-oxide (CNO; 1µM/side). Compared to vehicle (1xPBS), CNO significantly reduced binge-like ethanol consumption in hM4Di-expressing mice (4.22 vs 2.8 g/kg; $p<0.05$) without affecting intake in mice expressing control virus (3.80 vs 4.02 g/kg; $p>0.05$). Together, these data suggest that KOR activity in the BNST significantly contributes to binge-like ethanol consumption and the CeA may supply DYN tone to the BNST contributing to excessive ethanol intake.

Disclosures: H.L. Haun: None. **T. Kash:** None. **H.C. Becker:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.16/W16

Topic: G.08. Drugs of Abuse and Addiction

Support: AA026642
AA020930
AA023288

Title: Selective reduction of binge-like ethanol consumption through modulation of KV3 potassium channels in mice

Authors: *R. CANNADY[1], P. J. MULHOLLAND[1], C. H. LARGE[2];
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Abstract: Ethanol experience may influence maladaptive changes in the Kv3 voltage-gated potassium channel family. We have previously demonstrated that ethanol consumption and dependence is associated with covarying expression of Kv3 channel gene transcripts, including *Kcnc1* and *Kcnc3* in mice. Studies have also shown that knockdown of Kv3 channel subunits produces hypersensitivity to ethanol. Moreover, these channels are enriched in fast-spiking interneurons which have recently been implicated in modulating reward-seeking and goal-directed behavior. To date, however, no study has examined if Kv3 channels regulate ethanol consumption. Accordingly, we examined the effect of modulating Kv3 channels on ethanol consumption using a modified Drinking-in-the-Dark (DID) procedure to evoke binge-like

drinking behavior. Male C57BL/6J mice had limited (2hr) access to 20% ethanol or 1% sucrose (Mon-Thur) for 2 weeks. On the final drinking day, mice were administered the novel Kv3 channel positive modulator, AUT3, prior to access to ethanol or sucrose. AUT3 administration significantly reduced binge-like ethanol consumption. To test whether reduced ethanol consumption by AUT3 was specific to ethanol, a dose-response study was conducted in mice that consumed 1% sucrose. AUT3 did not reduce sucrose consumption at the tested doses; demonstrating specificity to reduce ethanol consumption. To rule out contributions of augmented locomotor activity, mice were tested in an open field after AUT3 administration. Total distance traveled in an open field was not significantly altered by AUT3 administration. Taken together, these preliminary findings indicate that Kv3 channels are a potential therapeutic target for selective reduction ethanol consumption. Ongoing studies will examine Kv3 channel contributions to escalated ethanol consumption in preclinical models of dependence and determine whether Kv3 channel modulation can alter dependence-induced interneuron dysfunction.

Disclosures: R. Cannady: None. **P.J. Mulholland:** None. **C.H. Large:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.17/W17

Topic: G.08. Drugs of Abuse and Addiction

Support: Supplement to R01 DA036572-01 (MDB)
T32GM008076 (NQC)

Title: Efficacy of selective GluK1-containing kainate receptor antagonism to modulate alcohol consumption and ethanol-related phenotypes in mice

Authors: *N. QUIJANO CARDÉ[1], E. PEREZ[2], H. KRANZLER[3], M. DE BIASI[3];
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Abstract: Alcohol use disorder (AUD) is a serious neuropsychiatric condition affecting millions of people worldwide. The heterogeneity of the disease underscores the need to expand the number of pharmacotherapeutics to treat AUD patients. Topiramate (TPM) is an antiepileptic drug that has been shown to modulate ethanol drinking patterns in humans. TPM has many pharmacological targets and affects many cellular processes and, therefore the molecular target for its effects on AUD is not clear. Among several mechanisms, TPM acts as a non-selective antagonist of kainate receptors containing the GluK1 subunit (GluK1*KARs), which is encoded by GRIK1 in humans. Interestingly, pharmacogenetic studies have shown that a single nucleotide polymorphism (SNP, rs2832407) in GRIK1 exerts an influence on the predisposition to develop

alcohol dependence and modulates the efficacy of topiramate treatment to reduce drinking. Thus, our study examined the ability of LY466195-mediated selective inhibition of GluK1*KAR to modulate responses to alcohol in a mouse model of alcohol dependence. Our results indicate that selective GluK1*KAR inhibition reduces ethanol intake and preference in mice undergoing short-term (24-h) and protracted (1 week) withdrawal in a dose-dependent manner. In mice undergoing short-term withdrawal, 20 mg/kg LY466195 treatment was sufficient to attenuate the manifestation of physical signs of withdrawal. Interestingly, we observed that chronic ethanol exposure in the intermittent two-bottle choice drinking paradigm affects the rewarding properties of ethanol as measured in the conditioned place preference (CPP) paradigm and with *in vivo* accumbal microdialysis. While mice chronically treated with ethanol in the I2BC failed to acquire/display ethanol CPP (1.5 g/kg ethanol), an acute administration of LY466195 (20 mg/kg) was sufficient to rescue the response observed in alcohol-naïve mice. We also found that LY466195 injection normalized dopamine responses to acute ethanol injection in mice undergoing short-term withdrawal from the I2BC. In summary, our data suggest that GluK1*KARs play an important role in modulating the reinforcing properties of ethanol that maintain addiction. Overall, our findings support the hypothesis that GluK1*KARs represent an attractive pharmacological target for the treatment of AUD.

Disclosures: **N. Quijano Cardé:** None. **E. Perez:** None. **H. Kranzler:** None. **M. De Biasi:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.18/W18

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant P50 AA10761
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NIH grant T32 AA007474
VA Medical Research (BX000813)

Title: Acute TLR3 activation with a poly I: C challenge results in a transient increase in ethanol intake in C57BL/6J male mice

Authors: *A. GANO[1], C. KING[1], H. C. BECKER[2];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]Charleston Alcohol Resch Ctr., Med. Univ. South Carolina, Charleston, SC

Abstract: Immune system activation has been implicated in the progression of alcohol addiction. Recently, it has been shown that Toll-like Receptor 3 (TLR3) stimulation increases ethanol consumption. The present experiments were conducted to examine the effects of acute immune challenge on ethanol self-administration behavior in adult male C57BL/6J mice via systemic administration of the TLR3 agonist polyinosinic:polycytidylic acid (poly I:C). In Experiment 1, we assessed the effects of an acute poly I:C challenge on home-cage drinking using a limited access two-bottle choice drinking paradigm. Mice were single-housed and given 2-hr access to 15% (v/v) ethanol and water starting three hours into the dark cycle, five days a week. After stable baseline intake was established, mice (n = 10-20/group) were given an injection (IP) of either vehicle (saline) or poly I:C (2, 5, 10 mg/kg). After a 48-hr recovery period, ethanol intake was measured for a week. Results indicated that all poly I:C doses increased ethanol intake on the first day of access, with the 5 mg/kg dose significantly elevating ethanol intake. However, this increase was transient, returning to baseline levels over several days. In Experiment 2, we utilized operant conditioning to assess the effects of an acute poly I:C challenge on ethanol oral self-administration. Mice were trained to respond on an FR4 schedule for access to 20 µl ethanol (20% v/v) infusions in daily 20-min sessions. After stable baseline ethanol responding/intake was established, mice (n = 12/group) were administered a 5 mg/kg poly I:C challenge or given a vehicle (saline) injection. After a 48-hr recovery period, mice were tested for oral ethanol self-administration in daily 20-min sessions. As compared to controls, animals challenged with poly I:C displayed an increase in ethanol intake similar to that observed in Experiment 1, which likewise resolved itself across the test week. Collectively, these data indicate that an acute poly I:C challenge produces a transient increase in ethanol consumption in different drinking paradigms. Ongoing studies are examining whether an immune challenge such as poly I:C injection further escalates ethanol intake in mice rendered alcohol-dependent.

Disclosures: A. Gano: None. **C. King:** None. **H.C. Becker:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.19/W19

Topic: G.08. Drugs of Abuse and Addiction

Title: The loss of environmental enrichment does not enhance alcohol self-administration

Authors: *X. M. HENRY, B. S. BURRELL, M. W. BARBEL, S. M. THOMPSON, D. N. TAPP, M. S. MCMURRAY;
Psychology, Miami Univ., Oxford, OH

Abstract: Addiction is an extremely prevalent disease that affects people from all aspects of life. Acute and chronic stressors are known contributors to both the development and maintenance of

addiction. In clinical settings, environmental/social factors, such as loss of job or spouse, often motivate drug use. Previous research suggests that animals continuously housed in enriched environments seek and consume drugs at lower rates than isolate-housed animals, possibly due to lower levels of stress. However, environmental situations are not always stable over the lifespan, and the loss of environmental enrichment could also affect an animal's motivation to seek and consume drugs. Therefore, we hypothesized that the loss of environmental enrichment will enhance alcohol self-administration. To address this, animals were assigned to either Continuous Enrichment, No Enrichment, or Enrichment Loss conditions. Enrichment was achieved by group housing and daily rotation of enrichment items, including balls, wheels, tubes, Nylabones, and chains. Animals arrived on postnatal day 25 and were immediately put into their respective environments (enriched or not). Animals in the Enrichment Loss condition were transferred to isolate housing on postnatal day 50. To determine if anxiety-like behaviors varied between conditions, elevated zero maze and open field testing occurred on postnatal days 67 and 68. Preference for alcohol was then assessed using a two-bottle choice, drinking-in-the-dark paradigm on postnatal days 69-79 (10% ethanol). Lastly, on postnatal days 80-81, animals completed a sucrose preference test as a measure of anhedonia. Results indicated that there were no differences due to enrichment status in the levels of anxiety-like behavior in the elevated zero maze and open field test, nor were there differences in sucrose preference. Animals in both the Continuous Enrichment and Enrichment Loss groups increased alcohol preference across days of access, while the No Enrichment group did not increase alcohol self-administration or preference. Lastly, the Continuous Enrichment group preferred alcohol more than the No Enrichment group, especially on later days of testing (postnatal days 77-79). Our results indicate that the loss of environmental enrichment may not be associated with increased alcohol intake. These findings suggest that environmental stressors alone may not be sufficient to increase alcohol intake or preference.

Disclosures: X.M. Henry: None. **B.S. Burrell:** None. **M.W. Barbel:** None. **S.M. Thompson:** None. **D.N. Tapp:** None. **M.S. McMurray:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.20/W20

Topic: G.08. Drugs of Abuse and Addiction

Support: Professional Development Grant, Lycoming College
Joanne and Arthur Haberberger Fellowship, Lycoming College
George B. Gaul Endowed Student-Faculty Research Program, Lycoming College

Title: Caffeine-induced increases in the reinforcing effects of alcohol are independent of activity at the dopamine D_2 receptor

Authors: G. BARKELL, *S. E. HOLSTEIN;
Psychology, Lycoming Col., Williamsport, PA

Abstract: Growing rates of use of alcohol mixed with caffeinated energy drinks is concerning given prior research suggesting that caffeine may be substantially increasing alcohol intake. In previous experiments, we have found that moderate doses of caffeine (5-10 mg/kg) increase both operant responding and motivation for a sweetened alcohol solution. These results may suggest that caffeine increases drug-seeking or consummatory behaviors for alcohol by enhancing the positive reinforcing effects of alcohol. The purpose of the current study was to examine whether caffeine may be enhancing the reinforcing efficacy of alcohol through its actions at the adenosine A_{2A}-dopamine D_2 receptor complex. Male Long Evans rats (n = 8) were administered 5 mg/kg caffeine, 0.01 mg/kg eticlopride (a dopamine D_2 receptor antagonist), or a combination of the two drugs 30 min prior to the operant self-administration session. A separate experiment evaluated the effects of caffeine, eticlopride, and the combination of these two drugs on motivation for alcohol using a progressive ratio schedule of reinforcement. Consistent with our previous studies, caffeine increased both alcohol-reinforced responding and motivation for alcohol; however, this increase in responding was at later time points in the operant conditioning session (10-15 min), suggesting that caffeine may be selectively increasing consummatory, rather than drug-seeking, behaviors. Although eticlopride alone did modestly reduce alcohol-reinforced responding, it did not attenuate the caffeine-induced increase in operant responding for alcohol. These results suggest that caffeine's reinforcement-enhancing effects may be independent of any caffeine-induced potentiation of dopamine D_2 receptor function via the A_{2A}-D_2 receptor complex.

Disclosures: G. Barkell: None. **S.E. Holstein:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.21/W21

Topic: G.08. Drugs of Abuse and Addiction

Support: DGAPA-PAPIIT Grant IN215218 to OPG
DGAPA-PAPIIT Grant IA205218 to MMD
DGAPA-PAPIIT Grant IN217918 to AERC

Title: Effects of maternal separation during light or dark phase of the cycle on CB1 and D2 receptor expression and alcohol consumption in adult male rats

Authors: *O. AMANCIO-BELMONT, A. BECERRIL-MELÉNDEZ, M. MÉNDEZ-DÍAZ, A. RUIZ-CONTRERAS, Ó. PROSPÉRO-GARCÍA;
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Abstract: Early life adversity is linked to the development of a number of psychiatric illnesses, including substance use disorder. Maternal separation (MS) is a model of early life stress and it has been associated with drug abuse and drug addiction. Dopaminergic and endocannabinoid (eCBs) systems have been implicated in reward processes, including drug intake and drug dependence. MS causes changes in the dopaminergic and eCBs in the prefrontal cortex (PFC) and nucleus accumbens (NAcc) that seem to facilitate alcohol consumption. Maternal care have diurnal variations. It is, therefore, of interest to understand if MS at different phases of the dark/light cycle differentially affects the dopaminergic and eCB systems in PFC, NAcc and amygdala in male rats.
Pregnant Wistar rats were obtained at gestational day 14-17 from our facilities (Facultad de Medicina, UNAM). The day of birth was designated as PND 0. MS was performed from PND 2 to PND 15, (8:00 -11:00 for MS in the light and 20:00 - 23:00 for MS in the dark). Rats were weaned on PND 21. Once the rats became adults (PND 60), all groups (n = 10 each group) were submitted to a voluntary alcohol (10% v/v) protocol for 10 days. Different groups of rats (n = 10 each group) were sacrificed when adult but with no exposition to alcohol whatsoever to dissect PFC, NAcc and amygdala to analyze the expression of cannabinoid 1 receptor and dopaminergic D2 receptor.
Results. Although MS increases alcohol consumption and preference regardless of the phase of the cycle, MS in the dark induced higher alcohol consumption and preference.

Disclosures: O. Amancio-Belmont: None. **A. Becerril-Meléndez:** None. **M. Méndez-Díaz:** None. **A. Ruiz-Contreras:** None. **Ó. Prospéro-García:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.22/W22

Topic: G.08. Drugs of Abuse and Addiction

Support: Miami University Department of Psychology

Title: Enhanced vulnerability to aversion-resistant alcohol drinking in female mice

Authors: *A. M. THOMAS, O. RAMSEY, S. MONROE, E. A. SNEDDON, A. K. RADKE;
Psychology, Miami Univ., Oxford, OH

Abstract: A key component of alcohol use disorder (AUD) is aversion-resistant drinking, or drinking despite negative consequences. In order to understand this phenomenon, aversion-resistant intake models of AUD have been developed in rodents. Preclinical research suggests that females may be more susceptible to addictive behaviors than males. The current study examined sex differences in a model of compulsive-like alcohol seeking. For all experiments, C57BL/6J mice were food restricted to 85% of their free-feeding weight then trained to respond for an alcohol reward on a fixed ratio 3 (FR3) schedule in an operant conditioning box. A sucrose fading procedure was used such that ethanol (EtOH) was added to the solution and sucrose was faded out, as follows: 10% sucrose + 10% EtOH, 5% sucrose + 10% EtOH, 10% EtOH. For Experiment 1, the concentration of EtOH was increased over several sessions: 15%, 20%, and 25%. Males and females did not differ in consumption of 10% EtOH, but females consumed significantly more at the 15%, 20%, and 25% concentrations than males. In Experiment 2, quinine hemisulfate was added to the 10% EtOH solution in increasing doses over 12 days: 0 μM, 100 μM, 250 μM, and 500 μM. Females continued to seek quinine-adulterated EtOH at all concentrations of quinine while males stopped responding at the 250 μM and 500 μM concentrations. To show that this compulsive behavior of drinking was specific to alcohol, a follow-up experiment was completed using the same protocol with a sucrose reward (2.5%) in place of EtOH. Both males and females suppressed their responding for 2.5% sucrose at all concentrations of quinine. Additionally, in a two-bottle preference test, males and females equally avoided these concentrations of quinine in water. For Experiment 3, responding was punished with a 0.25 mA footshock on the second nose poke of the FR3 sequence. Both males and females continued to respond for EtOH under these conditions. The results of this study provide insight into female alcohol drinking behaviors and suggest that females may be more susceptible compulsive-like alcohol seeking. Ongoing studies on this topic are examining the contribution of sex hormones and sex chromosomes to this behavior.

Disclosures: A.M. Thomas: None. **O. Ramsey:** None. **S. Monroe:** None. **E.A. Sneddon:** None. **A.K. Radke:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 078.23/W23

Topic: G.08. Drugs of Abuse and Addiction

Support: R00AA021782

Title: Subregion-specific effects of pituitary adenylate cyclase-activating polypeptide isoforms in the nucleus accumbens on ethanol drinking

Authors: *A. T. GARGIULO, B. E. PIRINO, G. R. CURTIS, P. SHAH, J. R. BARSON; Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Alcohol use disorder is widespread and multifaceted, but currently available pharmacotherapies are often not effective. Identifying novel molecules that affect ethanol drinking may thus lead to the development of more beneficial medications. Very limited evidence has suggested that pituitary adenylate cyclase-activating polypeptide (PACAP) can reduce ethanol drinking, but the brain regions and protein isoforms through which this occurs remain to be identified. Recent findings in our laboratory have suggested that one isoform, PACAP-27, could play an important role in this behavior. Notably, while the other isoform, PACAP-38, is more ubiquitously expressed in the brain and has been associated with stress-related behaviors, PACAP-27 is more selectively expressed and has not been linked to such behaviors. In the present study, we examined the effects of the two PACAP isoforms on ethanol drinking, testing them in the nucleus accumbens shell (NAcSh) and core (NAcC), regions associated with motivation and reinforcement. Adult male and female Long-Evans rats were trained to drink 20% ethanol or 5% sucrose in their home cage using the intermittent-access two-bottle choice paradigm (three 24-hour sessions per week). After establishing stable drinking, they were bilaterally cannulated into either NAcSh or NAcC. After recovery, they were injected bilaterally in a within-subject Latin-square design with PACAP-27 or PACAP-38 (25 pmol, 50 pmol), compared to saline vehicle (0.3 µl). In the NAcSh of ethanol-drinking rats (n = 10 males, 8 females), PACAP-27 significantly reduced ethanol drinking without affecting intake of simultaneously-available chow or water; PACAP-38 had no significant effects. In the NAcSh of sucrose-drinking rats (n = 10 males), PACAP-27 but not PACAP-38 led to a small, delayed suppression of sucrose drinking. In the NAcC of ethanol-drinking rats (n = 8 males), PACAP-38 but not PACAP-27 reduced ethanol drinking. Thus far, the data demonstrate that pharmacologically-relevant ethanol drinking can be significantly inhibited by endogenous PACAP-27, acting in the NAcSh, and by PACAP-38, acting in the NAcC. We propose that the PACAP isoforms should be further investigated for their utility as novel targets for the treatment of alcohol use disorder.

Disclosures: A.T. Gargiulo: None. **B.E. Pirino:** None. **G.R. Curtis:** None. **P. Shah:** None. **J.R. Barson:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

Location: Hall A

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Program #/Poster #: 078.24/W24

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH GRANT P20GM113109

Title: Intermittent alcohol exposure on differentially reared rats affects and their taste response to ethanol

Authors: *T. J. MOSER[1], T. J. WUKITSCH[1], M. CAIN[2];
[1]Psychological Sci., Kansas State Univ., Manhattan, KS; [2]Psychological Sci., Kansas Sate Univ., Manhattan, KS

Abstract: The incentive motivation for ethanol in differentially reared rats varies depending on the paradigm used. In operant paradigms, isolated condition (IC) rats respond more than enriched condition (EC) rats for ethanol, suggesting that IC rats are more motivated to consume ethanol. However, in a taste reactivity paradigm, IC rats exhibit less aversive and hedonic responses to ethanol than EC rats. This suggests that the isolation may not increase the liking component of incentive motivation. However, it is not clear if previous ethanol exposure will alter taste reactivity to ethanol in differentially reared rats and result in a change in liking. Our current study evaluates hedonic and aversive responding between male and female rats raised in Enriched (EC), Isolated (IC), and Standard (SC) conditions during adolescence while exposed to intermittent alcohol exposure (IAE). We hypothesized that overall IAE IC rats will exhibit lower aversive and higher hedonic responding compared to IAE ECs and SCs across a range of ethanol concentrations. Also, we hypothesized that IAE IC rats will have higher hedonic responding to sucrose than EC and SC IAE rats. Male and Female Long-Evans rats arrived at the lab on postnatal day (PND) 21 and were then randomly assigned to either EC, IC, or SC with their sex cohorts for a 30-day rearing period. During the rearing period, twelve injections of 20% ethanol (w/v; ip) were administered every other day through PND 50. Rats were implanted with intraoral fistulas that were routed through the mouth and anchored to the skull. After a week of recovery, rats underwent taste reactivity testing with a range of concentrations of ethanol and sucrose, presented in a counterbalanced order (H2O; 5%, 10%, 20%, 30%, & 40% ETOH; 0.1 M, 0.25M, & 0.5 M Sucrose). Videos were scored for behaviors frame by frame for sixty seconds, with standards provided by Grill and Norgren (1978). Preliminary results indicate that IAE does not significantly affect the amount of hedonic or aversive behaviors exhibited to different tastants. Consistent with our previous work, IC rats exhibited less aversive responses to ethanol compared to EC and SC rats. However, there were no differences between the rearing groups in hedonic responses. These findings indicate that taste reactivity to ethanol and sucrose do not change when exposed to ethanol via injections during adolescence, but that taste reactivity can be changed by the rearing environment.

Disclosures: **T.J. Moser:** None. **T.J. Wukitsch:** None. **M. Cain:** None.

Poster

078. Mechanisms Underlying Alcohol Consumption I

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Support: NIH:AA026685(CC)
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NIH:MH 096889 (TZB)
Hewitt Biomedical Foundation (JLB)

Title: A novel mouse model of the interaction between early life stress and vulnerability to ethanol dependence in C57BL/6J mice

Authors: *A. OKHUAROBO[1,2], J. L. BOLTON[3], I. IGBE[2], T. Z. BARAM[3], C. CONTET[1]; [1]Dept. of Neurosci., The Scripps Res. Inst., LA Jolla, CA; [2]Dept. of Pharmacol. and Toxicology, Univ. of Benin, Benin City, Nigeria; [3]Dept. of Anat. & Neurobio., Univ. of California-Irvine, Irvine, CA

Abstract: Vulnerability to ethanol dependence due to early life stress (ELS) is well established in humans. However, existing animal models of this interaction are not satisfactory, because they use modalities that yield sub-intoxicating blood ethanol levels, thereby excluding an important aspect of the human condition characterized by chronic excessive alcohol consumption and heavy intoxication. Hence, the present study aims to develop a more relevant mouse model of alcohol dependence vulnerability due to ELS and to study the vulnerability to negative affect due to this interaction in C57BL/6J male and female mice. We used a combination of the limited bedding and nesting (LBN) model of ELS, which yields fragmented maternal care from postnatal days 2 to 9 and long-lasting emotional and cognitive alterations in the offspring, with the chronic intermittent ethanol vapor inhalation (CIE) model of alcohol dependence, which yields voluntary ethanol intake escalation during limited-access two-bottle choice alcohol drinking sessions in adulthood. LBN rearing did not impact baseline alcohol consumption in males or females. However, males exposed to both LBN and CIE escalated their alcohol intake at an earlier stage of dependence induction than their counterparts raised under normal conditions. In contrast, CIE exposure did not affect voluntary ethanol consumption in females from the LBN and Control subgroups, possibly due to higher baseline ethanol intake compared to males. We also examined signs of negative affect after 2-3 weeks withdrawal from CIE. In the elevated plus-maze, CIE reduced open arm exploration and increased closed arm exploration selectively in male mice with ELS history while it did not affect their control-reared counterparts, nor females from either group. CIE increased digging activity in the digging test, reduced grooming activity in the splash test, and increased immobility time in the tail suspension test in both male and female mice regardless of their rearing conditions. In males, LBN reduced thermal nociception in the tail immersion test without altering mechanical nociception in the tail pressure test, and CIE increased mechanical nociception. This effect was not influenced by ELS history. Altogether, our data indicate that alcohol dependence vulnerability due to ELS can be modeled in C57BL/6J male mice by combining the LBN and CIE procedures, which results in accelerated drinking escalation to intoxicating levels and reveals an anxiogenic-like effect of alcohol withdrawal. This new model holds promises for the study of the molecular underpinnings and psychopathological consequences of alcohol dependence vulnerability induced by ELS.

Disclosures: **A. Okhuarobo:** None. **J.L. Bolton:** None. **I. Igbe:** None. **T.Z. Baram:** None. **C. Contet:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.01/W26

Topic: G.08. Drugs of Abuse and Addiction

Support: AA025590

Title: Low-dose ethanol activates hypothalamic POMC-expressing neurons

Authors: ***L. HOOD**, E. NAGY, J. PIÑA, M. F. OLIVE;
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Abstract: Hypothalamic neurons that express proopiomelanocortin (POMC) are important for regulating metabolism and experiencing satiety. The POMC peptide is also a precursor for endogenous opioids such as beta-endorphin, which are implicated in driving addiction and alcoholism. Increased endorphin levels are found in the nucleus accumbens, amygdala, and hypothalamus of rodents after both acute and chronic ethanol exposure. However, it remains unclear how ethanol acts on various endogenous opioid-containing circuits in the brain. The current study investigated the effects of voluntary ethanol intake on POMC neuron activity in the arcuate nucleus of the hypothalamus. Male and female transgenic mice expressing enhanced GFP (eGFP) under the POMC promoter were given free access to 20% ethanol for two hours during the dark phase of their light cycle in a paradigm titled drinking-in-the-dark (DID). After 3 consecutive days of habituation to this procedure, mice were given short access (20 min) to either 20% ethanol, 0.25% saccharin, or water. One hour after the 20-min access period, mice underwent transcardial perfusion, blood sampling for assessment of blood alcohol levels, and brain extraction for immunohistochemical analysis of c-fos expression. Ethanol intake and blood ethanol levels in males were 0.6±0.2 g/kg and 10±5 mM, respectively, and in females were 0.6±0.1 g/kg and 7±3 mM, respectively. The percentages of c-fos expressing POMC neurons were 19.2±2.5% in ethanol-consuming males and 17.3±4.3% in ethanol consuming females, which were significantly greater than those observed in saccharin or water consuming mice. A positive correlation between ethanol intake and the percent of c-fos+ GFP neurons was found. The spatial distribution of c-fos+ GFP neurons within the arcuate was also mapped using the passive clarity technique to image the entirety of the arcuate nucleus. These data suggest POMC-expressing neurons in the arcuate nucleus are a target of low-dose ethanol when voluntarily consumed.

Disclosures: **L. Hood:** None. **E. Nagy:** None. **J. Piña:** None. **M.F. Olive:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.02/W27

Topic: G.08. Drugs of Abuse and Addiction

Support: AA025590

Title: Concentration-dependent bidirectional effects of ethanol on POMC neuronal physiology

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Abstract: Alcohol abuse is a worldwide public health concern and leads to an estimated 90,000 alcohol-related deaths in the United States annually. Recent evidence suggests that alcohol may promote its euphoric and motivational effects, in part, by activating the endogenous opioid system. Further supporting the role of the endogenous opioid system in alcohol abuse, one of the most frequently utilized medications for treating alcohol use disorders to date is naltrexone, a broad spectrum opioid receptor antagonist. One particular circuit of the endogenous opioid system consists of pro-opiomelanocortin (POMC) producing neurons in the arcuate nucleus (ArcN) of the hypothalamus, which project heavily to reward-related areas. To identify the physiological effects of ethanol on POMC neurons, we utilized whole cell patch-clamp recordings of POMC neurons from POMC-EGFP mice and bath application of ethanol (5-40 mM) to identify alterations in *(1)* spontaneous baseline activity, *(2)* spike threshold/rheobase, *(3)* spiking characteristics or *(4)* intrinsic properties. Using whole-cell electrophysiology, we found that bath application of low concentrations of ethanol (10mM) increased the number of spikes in response to a depolarizing current in a majority of recorded cells. Additionally, in a majority of recorded cells, higher concentrations of ethanol (20-40mM) decreased the number of spikes in response to a depolarizing current. While ethanol changed the number of depolarization elicited spikes in a concentration-dependent manner, compared to control, rheobase was unaffected regardless of ethanol concentration. Additionally, spontaneous POMC activity, measured by spontaneous excitatory post-synaptic potentials (EPSPs) at rest were also unchanged in response to ethanol. Interestingly, 5mM ethanol had no effect on the number of spontaneous EPSPs, but significantly decreased the EPSP amplitude. Together, these results suggest that ethanol has concentration-dependent modulatory effects on POMC neuronal physiology. To our knowledge, these are the first studies to characterize the physiological effects of ethanol on POMC-neurons of the hypothalamus and may lend insight into treating alcohol use disorders.

Disclosures: J.M. Leyrer: None. **E. Nagy:** None. **M. Olive:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.03/W28

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
NIH Grant DA035958

Title: Low dose alcohol enhances dopamine release in the nucleus accumbens via alpha6-containing nicotinic receptors on gabaergic inputs from the ventral tegmental area

Authors: E. ANDERSON[1], A. STOCKARD[1], S. J. VOGEL[2], J. BRUNDAGE[1], A. PAYNE[1], D. OBRAY[1], M. GAO[3], J. MCINTOSH[4], A. M. LEE[5], J. YORGASON[1], S. SUDWEEKS[1], J. WU[6], ***S. C. STEFFENSEN**[1];
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Abstract: The prevailing view is that enhancement of dopamine (DA) transmission in the mesolimbic system consisting of DA neurons in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc) underlies the rewarding properties of ethanol (EtOH) and nicotine (NIC). Although the dogma is that EtOH enhancement of DA neural activity contributes to enhancement of DA transmission, DA neurons are not sensitive to rewarding levels of EtOH. However, VTA GABA neurons are sensitive to low-dose EtOH. We have shown previously that EtOH modulation of DA release in the NAc is mediated by α6-containing nicotinic receptors (α6*-nAChRs), that α6*-nAChRs mediate low-dose EtOH effects on VTA GABA neurons and EtOH preference, and α6*-nAChRs may be a molecular target for low-dose EtOH. Thus, the most sensitive target for reward-relevant EtOH modulation of mesolimbic DA transmission and the involvement of α6*-nAChRs in the mesolimbic DA reward system remains to be elucidated. The aim of this study was to evaluate EtOH effects on VTA GABAergic input to CINs and DA release in the NAc. Using DIO channel rhodopsin-2 (ChR2) viral injections into the VTA of VGAT Cre mice, we found that VTA GABA neurons send an inhibitory projection to CINs, replicating what has been demonstrated by others. Low-dose EtOH (IC_{50} = 10 mM) decreased optically-evoked IPSCs (oIPSCs) on CINs and enhanced (EC_{50} = 10 mM) CIN-mediated spontaneous DA release. Surprisingly, oIPSCs on CINs were not blocked by typical GABAA receptor (GABAAR) antagonists, but by GABAR rho-1 antagonists, suggesting involvement of atypical GABARs on CINs that are postsynaptic to VTA GABAergic input. This is supported by immunohistochemistry studies showing rho-1 expressed in CINs. The α6-conotoxin MII blocked the effects of EtOH on spontaneous DA release and optically-evoked DA release in choline

acetyltransferase (ChAT) ChR2 mice. Chronic administration of NIC enhanced EtOH consumption in the drink-in-the-dark procedure and EtOH preference in the CPP procedure and concomitantly enhanced expression of α6*-nAChRs in VTA GABA neurons, without affecting other nAChR subunits. Taken together, these findings suggest that VTA GABA neuron inhibitory input to CINs is modulated by α6*-nAChRs and sensitive to low-dose EtOH, which may underlie the rewarding properties of EtOH. Work is in progress to evaluate the effects of reduction of expression of α6*-nAChRs in VTA GABA neurons with α6-shRNA on DA release in the NAc and EtOH consumption and reward.

Disclosures: **E. Anderson:** None. **A. Stockard:** None. **J. Brundage:** None. **A. Payne:** None. **D. Obray:** None. **M. Gao:** None. **J. McIntosh:** None. **A.M. Lee:** None. **J. Yorgason:** None. **S. Sudweeks:** None. **J. Wu:** None. **S.C. Steffensen:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.04/W29

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA035958
NIH Grant AA020919
Beckman Scholar Program

Title: Evaluating the influence of sex and estrus cycle phase on dopamine dynamics in the mesolimbic pathway following acute ethanol administration

Authors: *M. PARSONS, J. OBRAY, E. BALDWIN, J. BOWMAN, R. JORGENSEN, S. STEFFENSEN;
Neurosci., Brigham Young Univ., Provo, UT

Abstract: The prevailing view is that enhancement of dopamine (DA) transmission in the mesolimbic DA system originating in the midbrain ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc) underlies the rewarding properties of alcohol. The rewarding properties of drugs of abuse including ethanol are felt to be mediated by an enhancement in DA transmission within this system. The role of sex in the well-known effects of ethanol on DA neural activity and release in the NAc has not been studied. We have found evidence of sex differences in ethanol effects on both tonic and phasic DA release in the NAc. Mainly, female rats evince a relatively shorter duration of ethanol enhancement of DA release followed by inhibition. Additionally, female rats seem to show decreased sensitivity to ethanol mediated reductions in evoked phasic DA release. Further, we have found evidence of differential variations in ethanol effects on DA release across the different phases of the estrus cycle. These

findings are important as they help us better understand sex and estrus differences in ethanol induced alterations of DA release. Additional experiments are underway to more fully characterize these effects.

Disclosures: M. Parsons: None. **J. Obray:** None. **J. Bowman:** None. **R. Jorgensen:** None. **S. Steffensen:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

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Program #/Poster #: 079.05/W30

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant AA023786
NIH grant P60-AA007611

Title: Impact of acute ethanol injections on medial prefrontal cortex neural activity

Authors: *M. MORNINGSTAR, B. MA, C. C. LAPISH;
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Abstract: The medial prefrontal cortex (mPFC) is a cortical brain region involved in the evaluation and selection of motivationally relevant outcomes and its function is impaired following alcohol use. In rodent models, doses as low as 0.75 g/kg yield deficits in cognitive functions. Deficits in decision-making following acute ethanol (EtOH) are thought to be mediated, at least in part, by decreases in mPFC firing. However, these data have been generated exclusively in anesthetized rodents. To eliminate the potentially confounding role of anesthesia on EtOH-evoked changes in mPFC firing, the present study investigated the effects of acute EtOH injections on mPFC neural activity in awake-behaving rodents. All recordings took place in adult, male Wistar rats (350-450 g). We utilized three groups: the first group received 2 saline injections during the recording. The second group received a saline injection followed 30 minutes later by a 1.0 g/kg EtOH injection. The last group received a saline injection followed 30 minutes later by a 2.0 g/kg EtOH injection. All recordings took place in a habituated open field. One week following the awake-behaving recording, an anesthetized recording was performed using one dose of saline followed 30 minutes later by one dose of 1.0 g/kg EtOH in order to replicate previous studies. Raw data was spike sorted utilizing Spyking-Circus and analyzed in MATLAB. Firing rates were normalized to a baseline period that occurred 5 minutes prior to each injection. A 5-minute time period 30 minutes following the injection was used to compare against baseline. In the preliminary results, groups were compared across the following conditions: awake-behaving: saline-saline (N = 122 neurons, N = 6 animals), saline- EtOH 1 g/kg (N = 160 neurons, N = 8 animals), anesthetized: saline-EtOH 1 g/kg without alcohol history

(N = 126 neurons, N = 6 animals), saline-EtOH 1 g/kg with alcohol history (N = 239 neurons, N = 8 animals). A main effect was detected across groups (Kruskal-Wallis, $X^2 = 36.03$, $p < 0.01$) and was followed up on using a Dunn-Sidak multiple comparison test. The results of the Dunn-Sidak test indicate in both groups that our anesthetized condition reliably produced decreases in firing rates following an injection of 1.0 g/kg EtOH. However, in the awake-behaving condition the 1.0 g/kg dose of EtOH produced a significant elevation of firing rates compared to saline controls. This suggests that the cognitive deficits associated with EtOH exposure may be due to mechanisms other than broad reductions in firing rate.

Disclosures: M. Morningstar: None. **B. Ma:** None. **C.C. Lapish:** None.

Poster

079. Alcohol's Effects on the Brain

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Program #/Poster #: 079.06/W31

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA Grant U01 AA020912-08S1
NIAAA Grant P50 AA022538

Title: Binge drinking alters orthodenticle homeobox 2 gene and protein expression in the ventral tegmental area

Authors: *C. COLES, A. W. LASEK;
Anat. and Cell Biology, Psychiatry, and Ctr. for Alcohol Res. in Epigenetics, Univ. of Illinois At Chicago, Chicago, IL

Abstract: The ventral tegmental area (VTA) is an important brain region involved in the development of both alcohol use and affective disorders such as depression. Studies have shown that depletion of orthodenticle homebox 2 (OTX2) in the mouse VTA during the juvenile period leads to increased susceptibility to depression-like behaviors in adulthood. Since there is comorbidity between alcoholism and depression, we hypothesize that VTA OTX2 may play a role in binge drinking. The purpose of this study was twofold: 1) to measure VTA *Otx2* gene and protein expression after binge-like ethanol consumption and 2) determine if viral-mediated knockdown of VTA OTX2 during adulthood regulates binge-like drinking. For the gene and protein expression studies, male and female C57BL/6J mice underwent 4 days of the drinking in the dark (DID) procedure using a 20% ethanol solution or water as a control. The VTA was dissected from mice immediately after the 4th drinking session and 24 hours later. *Otx2* gene and protein levels were measured by qPCR and western blotting, respectively. *Otx2* mRNA was significantly decreased in both sexes immediately after ethanol drinking. At the 24-hour time point, the female ethanol-drinking group had significantly more *Otx2* mRNA than controls.

Interestingly, OTX2 protein levels were higher in females immediately after the ethanol-drinking session and in both sexes 24 hours after ethanol drinking compared with controls. These results demonstrate that binge-like drinking alters VTA *Otx2* gene and protein expression. Next, we investigated if knockdown of VTA *Otx2* using a lentiviral-delivered short hairpin (sh)RNA altered binge-like drinking in the DID test. Three weeks after stereotaxic microinjection of virus into the VTA, male and female mice went through 3 cycles of 4 days of DID, followed by 4 days of 2% sucrose consumption to measure ethanol consumption and anhedonia, respectively. Although there was a trend for increased ethanol drinking in females expressing *Otx2* shRNA compared with the control shRNA, we did not observe a significant difference in ethanol or 2% sucrose consumption in either sex. Future experiments will determine if manipulation of *Otx2* expression during the juvenile period plays a role in binge drinking in adulthood. Supported by NIAAA (U01 AA020912-08S1 and P50 AA022538).

Disclosures: C. Coles: None. **A.W. Lasek:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.07/W32

Topic: G.08. Drugs of Abuse and Addiction

Support: PHS NIH RO1 AA023410
PHS NIH R21 AA024036

Title: Chloride and calcium dynamics underlying ethanol-induced interneuronopathy

Authors: *S. M. LEE[1], R. J. DING[2], A. G. J. SKORPUT[3], P. W. L. YEH[1], H. H. YEH[1];
[1]Dept. of Mol. and Systems Biol., Geisel Sch. of Med. at Dartmouth Col., Hanover, NH;
[2]Dartmouth Col., Hanover, NH; [3]Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Alcohol consumption during pregnancy places the unborn child at risk for fetal alcohol spectrum disorders (FASD). While the diagnosis and management of FASD takes place postnatally, the underlying pathoetiology remains incompletely understood but is likely embryonic. In mice, prenatal exposure to ethanol disrupts the pattern of tangential migration of cortex-bound primordial GABAergic interneurons, and this has been postulated to contribute to long-term excitatory/inhibitory imbalance within the cortical circuits and impairments in executive function. There is evidence that ethanol interacts with $GABA_A$ receptors that are tonically activated by ambient GABA to exert this effect but the cellular and subcellular mechanisms are largely unexplored. Here we begin to investigate the mechanisms underlying the migration of GABAergic interneurons and how this might be disrupted following ethanol exposure.

First, we asked whether ethanol exposure enhances $GABA_A$ receptor-induced depolarization. Perforated patch clamp recordings were performed on tdTomato-positive GABAergic interneurons from acute E14.5 Nkx2.1Cre/tdTomato slices to measure $GABA_A$ receptor reversal potential (E_{GABA}) before and during 6.5mM ethanol (30mg/dl or 0.03% equivalent) exposure. Ethanol exposure shifted E_{GABA} to being more depolarized compared to control. Ethanol also potentiated the amplitude of current responses to 50μM GABA. Pretreatment of embryonic slices with 20μM bumetanide blocked the ethanol-enhanced GABA depolarization. Acute exposure to 18mM ethanol (80mg/dl or 0.08% equivalent) also shifted E_{GABA} to being more depolarized and the ethanol-induced change in GABA response amplitude was blocked by 20μM bumetanide. Second, we asked whether the ethanol-enhanced GABA chloride flux is linked to a rise in intracellular calcium via voltage-gated calcium channels, since calcium is implicated in regulating neuronal migration and cytoskeletal dynamics. To this end, pregnant dams from E13.5 to E16.5 were assigned liquid diet with 5% ethanol, nifedipine, 5% ethanol with nifedipine, or isocaloric control food. The addition of the calcium channel blocker nifedipine prevented the ethanol-induced aberrant tangential migration implicating calcium channels in the ethanol-induced effect. Ongoing experiments are employing the calcium indicator Fluo-5F to monitor ethanol-induced changes in intracellular calcium and correlate this with changes in growth cone dynamics.
The present study sets the stage for filling mechanistic gaps that link chloride homeostasis to calcium dynamics in regulating the migration of immature GABAergic cortical interneurons.

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Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.08/W33

Topic: G.08. Drugs of Abuse and Addiction

Support: Swedish medical research concil
Swedish Brain Fondation
LUA/ALF grant

Title: An acetylcholine-dopamine interaction in the rat nucleus accumbens and its tentative involvement in ethanol's dopamine-liberating effect

Authors: *A. ANDRÉN[1,2], L. ADERMARK[1], B. SÖDERPALM[1,2], M. ERICSON[1];
[1]Addiction Biol. Unit, Dept. of Psychiatry and Neurochemistry, Univ. of Gothenburg, Gothenburg, Sweden; [2]Beroendekliniken, Sahlgrenska Univ. Hosp., Gothenburg, Sweden

Abstract: Alcohol use disorder is a chronic, relapsing brain disorder associated with serious medical consequences leading to preterm death. Although few in number, cholinergic interneurons (CIN) have arisen as an important cell population within the nucleus accumbens (nAc) that may exert a regulatory impact on dopamine (DA) neurotransmission locally. A defect in CIN have been suggested to be involved in psychiatric diseases such as alcohol addiction. The mechanisms through which endogenous cholinergic activity modulates DA release in response to ethanol administration and its role in development of addiction is not known. In this project, the aim was to study if acetylcholine (ACh) can influence DA release locally in the nAc and if so, through which receptor population(s) this effect is mediated. Further, we wanted to determine the role of ACh in ethanol-induced DA elevation.Using reversed in vivo microdialysis, the acetylcholinesterase inhibitor physostigmine was administered locally in the nAc of male Wistar rats followed by addition of either the muscarinic ACh receptor inhibitor scopolamine or the nicotinergic ACh receptor inhibitor mecamylamine. Subsequently, ethanol was perfused following local pretreatment with scopolamine or mecamylamine, using the same methodology. An immunotoxin, anti-ChAT-saporine, was infused locally into the nAc of a subset of male Wistar rats to selectively lesion CIN, followed by local ethanol administration via reversed in vivo microdialysis. Local administration of physostigmine induced a DA elevation within the nAc, an effect blocked by scopolamine but not by mecamylamine. Local administration of ethanol increased DA levels. Scopolamine pretreatment non-significantly attenuated the ethanol-induced DA elevation, whereas pretreatment with mecamylamine had no effect. Preliminary results indicate a minor attenuation of the DA elevation observed after local administration of ethanol in toxin-treated animals, as compared to sham-treated controls. Taken together, these results suggest that ACh increases extracellular DA levels in nAc in vivo, an effect mediated by muscarinic ACh-receptors and not by nicotinic ACh-receptors. Considering that scopolamine moderately attenuated ethanol-induced DA output and that lesioning of CIN appeared to hamper DA release in response to ethanol, ACh release from CIN within the nAc may be partially involved in ethanol-induced DA release in nAc.

Disclosures: **A. Andrén:** None. **L. Adermark:** None. **B. Söderpalm:** None. **M. Ericson:** None.

Poster

079. Alcohol's Effects on the Brain

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Program #/Poster #: 079.09/W34

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA025110
NIH Grant AA023288

Title: Induction of alcohol dependence in C57Bl/6J mice alters prelimbic prefrontal cortical GCaMP6f activity in response to alcohol-predictive cues in the 2CAP task

Authors: K. A. CLAYTON[1], F. A. KHAN[2], C. C. LAPISH[3], P. J. MULHOLLAND[4], *J. A. RINKER[5];
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Abstract: According to recent estimates by NIAAA, alcohol use disorder (AUD) affects over 15 million people over the age of 18. The neurobiological underpinnings of AUD are not completely understood, therefore in order to better inform treatment strategies, we must first understand how alcohol use and dependence alters normal neural processing. The prelimbic-prefrontal cortex (PL-PFC) has been shown to play a large role in decision-making processes, and alterations in activity of this brain region may lead to poor, or inflexible, decision making. The PL-PFC is responsive to cues that are associated with, and predict, access to a reinforcer. While some evidence has shown that the PL-PFC shows increased activity after the development of alcohol dependence or in alcohol-preferring strains of rats, the responsivity of the PL-PFC to alcohol-related cues has not been well characterized in rodent models of alcohol dependence. Using fiber photometry and the genetically encoded calcium indicator (GECI) GCaMP6f expressed in PL-PFC pyramidal projection neurons, we characterized the neural response to alcohol- or water-associated cues and consumption of each reinforcer using a modified 2-cued access protocol (2CAP). In this protocol, C57BL/6J mice were exposed to either a solid cue light for 3s that predicted the availability of 15% ethanol (v/v) via a retractable sipper tube located directly under the cue light or a blinking cue light that predicted the availability of water, in a randomized fashion within the same session. This allowed for the direct comparison of PL-PFC activity in response to each cue and reinforcer consumption before and after the induction of dependence using the chronic intermittent ethanol (CIE) vapor exposure protocol. We hypothesized that mice would show an increased preference for and neuronal response to cues post-dependence. Results from initial experiments show that there was a significant increase in calcium signal in response to the alcohol cue compared to the water cue during initial training (t=4.099, $p<0.0001$ during training week 1), which diminished across training sessions (t=0.6107, $p=0.54$ during training week 5). As expected, mice significantly increased licking for the ethanol reinforcer (t=3.373, $p=0.0082$) after the induction of dependence, and interestingly, the diminished calcium signal in response to the alcohol cue showed a strong trend toward rebound (t=1.954, $p=0.05$). These results indicate that over training, cues predicting alcohol become less salient to the animal, and the salience of this signal is restored after the induction of alcohol dependence.

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Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.10/W35

Topic: G.08. Drugs of Abuse and Addiction

Support: AA025582

Title: Interoceptive conditioning with low alcohol dose: Inactivation of the dentate gyrus partially disrupted low dose alcohol discrimination

Authors: *D. F. LOVELOCK, K. VAN VOORHIES, P. A. RANDALL, T. KASH, J. BESHEER;
Bowles Ctr. for Alcohol Studies, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: The subjective effects of alcohol are an essential driver of alcohol-seeking behaviors and these effects are present even at low doses. The neurobiological mechanisms activated in response to low doses of alcohol may be critical to understanding how and why compulsive alcohol seeking develops. The current study builds on our previous work that found A) a low dose of alcohol (0.8 g/kg, IG) could serve as an interoceptive cue controlling behavior in a Pavlovian drug discrimination procedure, and B) that this same low dose of alcohol induced immediate early gene (activity-regulated cytoskeleton-associated protein (Arc) and c-Fos) expression in the dentate gyrus (DG) of the hippocampus. We hypothesized that this activity in the DG was essential for the expression of alcohol interoceptive effects and therefore successful performance in the Pavlovian alcohol discrimination task. To test this, male and female Long Evans rats were trained on the Pavlovian discrimination procedure where the drug state (0.8 g/kg ethanol, IG) predicts whether a light cue will be followed by a sucrose reward. Once alcohol reliably controlled behavior, subjects received implantation of guide cannulae aimed at the. After recovery and demonstration of continued successful discrimination, testing began. Test sessions were interspersed with training sessions. During the test sessions, rats received a microinjection of muscimol + baclofen (GABAA + GABAB agonist) to inactivate the DG or vehicle. This procedure was repeated after four consecutive successful drug discrimination training days such that all subjects were tested under all four possible conditions (ethanol/water x muscimol+baclofen/vehicle). Overall baclofen + muscimol reduced goal-tracking when all subjects were analyzed together indicating a reduction in sensitivity to alcohol, but this effect was not found when subjects were separated by sex. Thus, when the DG was inactivated alcohol drug discrimination was reduced rather than completely disrupted, suggesting that interoceptive discrimination of a low dose of ethanol is partially but not exclusively regulated by this region. Unexpectedly, locomotor activity during discrimination was increased in females by baclofen + muscimol regardless of drug state whereas locomotion was unchanged in males. These findings

serve as a first step towards understanding brain region and circuit-specific contributions in processing interoceptive cues of a low dose of alcohol and pave the way for application of chemogenetic tools able to target specific cell types and projections that can more clearly elucidate how these cues come to control behavior.

Disclosures: D.F. Lovelock: None. **K. Van Voorhies:** None. **P.A. Randall:** None. **T. Kash:** None. **J. Besheer:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.11/W36

Topic: G.08. Drugs of Abuse and Addiction

Title: Serotonin 1A receptor-dependent modulation of alcohol-induced deficits in 5-HT/VGLUT3 innervation

Authors: *A. BELMER, K. BEECHER, S. E. BARTLETT;
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Abstract: A subpopulation of raphe 5-HT neurons expresses the vesicular glutamate transporter VGLUT3, and the co-release of glutamate and serotonin has been proposed to play pivotal role in encoding reward-related behaviors. Serotonin axons are identifiable by immunolabelling of either serotonin (5-HT) or the plasma membrane 5-HT transporter (SERT), with SERT labelling only partially overlapping with 5-HT staining. Studies investigating the specific distribution of VGLUT3 in SERT or 5-HT immunolabelled axons have led to inconsistent results. Therefore, we developed a method that combines immunohistochemistry, high resolution confocal imaging and 3D-reconstruction techniques to map and quantify the distribution of VGLUT3 immunoreactive boutons within 5-HT vs SERT- positive axons in various regions of the mouse forebrain, including the prefrontal cortex, nucleus accumbens core and shell, bed nucleus of the stria terminalis, dorsal striatum, lateral septum, basolateral amygdala and hippocampus. We further used this approach to map the changes in 5-HT/VGLUT3 innervation following 12 weeks of chronic exposure to binge-alcohol (20% v/v) intake in the drinking-in-the-dark paradigm. As compared to sucrose (5% w/v) controls, chronic binge-alcohol consumption produces profound changes in 5-HT/VGLUT3 innervation in various brain regions, and interestingly, these alterations were totally reversed by a chronic treatment with the 5-HT1A partial agonist tandospirone (3mg/kg/day, ip). This data suggests that 5-HT1A receptors play a pivotal role in serotonin axon plasticity and its alteration following chronic alcohol intake. We have now started to dissect the contribution of 5-HT1A auto- vs heteroreceptors and the circuitry involved in this process using biased agonists, brain cannulation and chemogenetics.

Disclosures: **A. Belmer:** None. **K. Beecher:** None. **S.E. Bartlett:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

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Program #/Poster #: 079.12/W37

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA009082
NIH R01AA024774

Title: Alcohol drinking reduces the number of myelinated axons in the prefrontal cortex in male adolescent rats

Authors: *B. A. REYES[1], T. LHAMO[1], A. AL-SIBAI[1], L. BENGSTON[2], A. SILVA-GOTAY[3], E. TAVARES[5], H. N. RICHARDSON[4], E. J. VAN BOCKSTAELE[6];
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Abstract: Alcohol binge drinking impairs myelin-associated proteins in the medial prefrontal cortex (mPFC) and is associated with changes in the integrity of the prefrontal white matter. Myelination provides rapid and efficient conductance in axons. Abnormality and changes in myelination impair information processing and cognitive performance as mPFC in involved in executive functions, emotional and cognitive behavior. While our previous research indicates that voluntary binge drinking reduces myelin fiber density and myelin-related proteins in the mPFC of males, the changes that occur at the ultrastructural level have not been studied. In the present study, we used electron microscopy to quantify the number of axons, g ratio, and myelin abnormalities in the adolescent male and female mPFC following two weeks of operant self-administration of sweetened alcohol or sweetened water (postnatal days 28-42). One day after drinking ended, brains were glutaraldehyde-fixed and further processed for ultrastructural analysis of myelin. Our preliminary results showed that voluntary binge drinking seems to increase the g-ratio, percentages of uncompacted and degenerating myelin in male and female rats; however, alcohol appeared to have more robust effects in male rats. Additionally, alcohol decreases the number of myelinated axons in the male and female rats, and this effect is more pronounced in the male. The present results suggest vulnerability of the myelinated mPFC axons to the adverse effects of alcohol exposure that may underlie impaired cognitive performance associated with early alcohol use in humans.

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Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.13/W38

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA 1F31AA025518
NIAAA R01AA018354
NIAAA R21AA022650
NCATS TR001106

Title: Studying alcoholism with diffusion weighted MRI with neurite orientation dispersion and density modeling: Effects of diagnosis and alcohol intoxication on axonal density

Authors: *K. K. YODER[1], E. J. CHUMIN[1], M. DZEMIDZIC[2], K. L. HILE[1], M. E. HALCOMB[1], M. H. PLAWECKI[3], S. J. O'CONNOR[3], S. M. MUSTAFI[1], Y.-C. WU[1]; [1]Radiology and Imaging Sci., [2]Neurol., [3]Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Although it is accepted that alcohol alters the membrane properties of cells, little is known about how chronic or acute alcohol exposure may alter the acute *in vivo* intracellular properties of neurons. To address this, we applied neurite orientation dispersion and density (NODDI) modeling to diffusion magnetic resonance imaging data (dMRI). We predicted that chronic and acute ethanol exposure would alter white matter integrity and neurite parameters. Twenty-one social drinkers (SD) and 13 nontreatment-seeking alcoholics (NTS) received a baseline dMRI using the HYDI sequence. A subset of 10 SD received two other dMRI HYDI scans: one during an intravenous (IV) saline infusion, and during an IV alcohol infusion calculated to maintain breath alcohol concentration at 80 mg% during dMRI. Parametric images were created for the NODDI parameters of orientation dispersion, intracellular volume fraction (ICVF), and isotropic volume fraction, and for the diffusion tensor (DT) metrics of fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity. Average values for each parameter were extracted from the bilateral centrum semiovale. Independent *t*-tests were used to examine group differences in baseline metrics from the full sample. In the subset of 10 SD, repeated measures ANOVA with post-hoc paired *t*-tests were used to examine differences across the three conditions. There were no group differences in any baseline NODDI or DT parameters. There was a main effect of infusion ($F(2,8)=15.6$; p&lt0.002) relative to baseline. Saline caused an increase in ICVF from baseline (*p*&lt0.02). Alcohol produced higher ICVF compared to both baseline *(p*&lt0.0001) and saline (*p*&lt0.004). There were no significant effects of either saline or alcohol on any other NODDI or DT parameters. Contrary to our prediction, chronic alcohol use

did not alter either NODDI or DT metrics. However, our novel data indicate that acute fluid load may alter white matter composition, which is conventionally believed to be insensitive to acute pharmacological challenges. The data also suggest that NODDI may be more sensitive than canonical DT metrics for detecting transient changes in white matter microstructure. ICVF reflects axonal density under the rigid-stick assumption of NODDI, with higher ICVF values indicating increased axonal density per imaging voxel. Thus, the present results suggest that acute changes in hydration and/or alcohol intoxication may acutely decrease the intracellular fluid volume. Next steps should include determining if the effect on ICVF is dose-dependent with respect to alcohol, and whether the osmolality of a solution mediates similar results.

Disclosures: K.K. Yoder: None. **E.J. Chumin:** None. **M. Dzemidzic:** None. **K.L. Hile:** None. **M.E. Halcomb:** None. **M.H. Plawecki:** None. **S.J. O'Connor:** None. **S.M. Mustafi:** None. **Y. Wu:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.14/W39

Topic: G.08. Drugs of Abuse and Addiction

Support: AA021449,
DA023248
DA044749
EB022911
MH113134

Title: Posterior cingulate cortical response to active avoidance mediates the relationship between punishment sensitivity and problem drinking

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Abstract: Many people drink to alleviate negative affect, an avoidance strategy which can lead to alcohol misuse. Individuals with heightened sensitivity to punishment are especially susceptible to problem drinking via this maladaptive coping mechanism. As imaging studies have focused on sensation seeking traits and approach behavior, the neural substrates underlying behavioral avoidance as well as their relationship with punishment sensitivity and alcohol use remain unclear. Here, we examined the cerebral correlates of inhibition of action to avoid a penalty in relation to both problem drinking and sensitivity to punishment (SP), as evaluated by the Alcohol Use Disorders Identification Test (AUDIT) and the Sensitivity to Punishment and Sensitivity to Reward Questionnaire, respectively. Seventy non-dependent drinkers performed a

reward go/nogo task with approximately 2/3 go and 1/3 nogo trials. Correct go and nogo responses were rewarded and incorrect responses were punished. The results showed that SP and AUDIT scores were each positively correlated with brain activations during inhibition of action and these activations overlapped in the posterior cingulate cortex (PCC). Thus, the PCC may represent a shared neural substrate for avoidance, punishment sensitivity, and at-risk drinking. Mediation analyses further suggested that PCC response to avoidance completely and bidirectionally mediated the relationship between SP and problem alcohol use. These findings substantiated the role of the PCC in behavioral avoidance and its link to at-risk drinking in punishment-sensitive non-dependent drinkers.

Disclosures: **T.M. Le:** None. **S. Zhornitsky:** None. **W. Wang:** None. **J.S. Ide:** None. **C.R. Li:** None.

Poster

079. Alcohol's Effects on the Brain

Location: Hall A

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Program #/Poster #: 079.15/W40

Topic: G.08. Drugs of Abuse and Addiction

Support: R01-AA013892
K08-AA023545
1R01AA026844

Title: Functional disruption in signaling between central (CNS) and peripheral nervous system in AUD individuals

Authors: *S. HWANG, R. DOUGLAS, R. SINHA, C. LACADIE, D. SEO;
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Abstract: Alcohol use disorders (AUD) are associated with neuropathology and autonomic nervous system (ANS) disruption, either of which contribute to AUD severity including high craving and relapse risk. Specific disruption of the ventromedial prefrontal cortex (vmPFC), marked by hyperactivity in neutral state and blunted response to stress and alcohol cue, has been shown in our previous studies. To further understand concurrent vmPFC and ANS responses and their association with craving, we used a combined functional magnetic resonance imaging (fMRI) and electrocardiogram (ECG) method in 23 AUD patients (AD;12 women) and 19 light drinkers (LD; 7 women). During sustained exposure to stress (S), alcohol (A) and neutral (N) condition pictures, we examined neural response to the visual provocation in a 3T MRI Scanner. Concurrently, we collected ECG along with alcohol craving, stress and arousal ratings. We analyzed fMRI data using BioImageSuite and AFNI and processed ECG through MATLAB and Kubios software for heart rate (HR), low-frequency power (LF) and approximate entropy (ApEn;

autonomic irregularity indicating ANS disruption). We found AD, compared to LD, showed greater emotional and ANS disruption marked by increased craving, HR and ApEn. Higher craving in AD during baseline and provocation was found across conditions (S: $t=3.72$, A: $t=3.74$, N: $t=3.44$; $ps<0.01$) with higher arousal specific to alcohol provocation ($t=2.18$, $p<0.05$). Higher overall HR (S: $t=2.36$, A: $t=2.18$, N: $t=2.07$; $ps<0.05$) and baseline ApEn (S: $t=2.35$, A: $t=2.49$, N: $t=2.24$; $ps<0.05$) were also observed in AD across conditions. In addition, a significant association was found between craving and ANS function in AD, as basal HR correlated with time-related increased craving during alcohol cue ($r=.485$, $p<0.05$). During stress, higher craving associated with greater LF power throughout the condition ($r=.473$, $p<0.05$). However, this pattern was not observed in LD. Our results revealed greater basal state disruption in AD compared to LD, including greater autonomic irregularity, higher HR and increased craving. To corroborate, neuroimaging results further indicated that AD show vmPFC hyperactivity during neutral basal state, which predicted higher HR during stress ($p<0.05$). The current study suggests basal functional disruption in the CNS and its periphery (ANS) in AD predict behavioral correlates of high craving. Higher craving in AD may associate with basal state differences in autonomic response and neutral state hyperactivity in the brain under vulnerable conditions, such as alcohol-related environments. Continuous exposure to such cues may increase susceptibility in AD to addiction severity.

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Poster

079. Alcohol's Effects on the Brain

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 079.16/W41

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA R01AA021505 (JW)
NIAAA U01AA025932 (JW)

Title: Drug-induced synaptic plasticity from striatal medium spiny neurons to cholinergic interneurons and behavioral inflexibility

Authors: H. GANGAL, Y. CHENG, X. WANG, J. LU, D. ARGEYLAN, X. XIE, L. N. SMITH, J. WANG;
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Abstract: Increasing evidence indicate that acetylcholine levels in the dorsal striatum significantly increase in a reversal learning task. Striatal acetylcholine mainly arises from cholinergic interneurons (CINs), which are tonically active and constitute about 2% of the total

neural population in this area. The striatal principal neurons are GABAergic medium spiny neurons (MSNs), which express either dopamine D1 receptors (D1Rs) or D2Rs. It has been reported that MSNs make synaptic connections with CINs. We thus hypothesize that MSNs inhibit CIN activity. Using slice electrophysiology, we found that selective optogenetic excitation of D1R-MSNs or D2R-MSNs induced inhibitory postsynaptic currents (IPSCs) in striatal CINs, with a greater magnitude for D1R-MSNs than D2R-MSNs. A rabies-mediated retrograde monosynaptic neural tracing study found that D1->CIN connections were significantly stronger than D2->CIN connections. The same rabies study also revealed that CIN-innervating D1R-MSNs projected to the substantia nigra pars reticulata (SNr), which forms the direct pathway and controls reinforcement of addictive drugs. Next we examined how exposure to addictive substances, alcohol and cocaine, altered GABAergic inputs on CINs and CIN firing activity. We found that the spontaneous IPSC frequency and the D1->CIN IPSC amplitude were increased, whereas the spontaneous firing rate of CINs was significantly reduced in drug-administered animals, as compared to their water or saline controls. Importantly, we found that excessive alcohol consumption or repeated cocaine self-administration impaired reversal learning of operant sucrose and food self-administration. Our data suggest that drug-induced inflexibility may be mediated by the aberrant plasticity at D1->CIN connections.

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Poster

079. Alcohol's Effects on the Brain

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Topic: G.08. Drugs of Abuse and Addiction

Support: AA023786
P60-AA007611

Title: Neural encoding in medial prefrontal cortex during aversion resistant drinking in rodent models of alcohol use disorder

Authors: *N. M. TIMME[1], D. N. LINSENBARDT[1], M. TIMM[2], T. GALBARI[1], E. CORNWELL[1], C. C. LAPISH[1];
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Abstract: Aversion resistant drinking (i.e., continuing to drink alcohol despite negative consequences) is a defining feature of advanced stage alcohol use disorder (AUD) where positive treatment outcomes become less likely. To understand the changes in brain function that underlie this condition, the current experiments measured neural activity in a rat model of aversion

resistant drinking. In vivo electrophysiological recordings were performed on animals consuming alcohol alone or alcohol paired with an aversive stimulus (quinine). A control strain (Wistar) and a line selectively bread to prefer alcohol (alcohol preferring (P) rats) each underwent a 24-hour intermittent access protocol with 20% alcohol solution for several weeks to become acclimated to the taste and effects of alcohol. Next, the animals were trained to consume 10% alcohol in a simple 2-way cued access drinking task. Animals were then implanted with movable multieletrode shanks or tetrodes in mPFC. Behavioral and electrophysiological data were gathered during task maintenance (regular alcohol, no aversive stimuli) and in task sessions where the alcohol was adulterated with 0.1 g/L quinine (aversive stimuli). At this quinine concentration, we found that P rats continued to consume alcohol in the task, but preferred unadulterated alcohol when given the choice (i.e., they exhibited aversion resistant drinking). Conversely, Wistars decreased consumption at this same quinine concentration, though they continued to try to drink the solution throughout the task. Using electrophysiology in regular alcohol sessions, we found that the encoding of the alcohol cue and the decision to drink was reduced in P rats relative to Wistars, indicating a diminished role for mPFC in the decision to drink in this rodent model for genetic risk for AUD. Ongoing experiments will assess neural encoding of the decision to drink with quinine adulterated alcohol. These experiments indicate that quinine adulteration in this task with these rodent strains models aversion resistant and aversion sensitive drinking. Furthermore, these data indicate that mPFC plays an important role in the decision to drink in non-aversive scenarios. Ongoing studies will directly assess neural encoding in mPFC in aversion resistant and aversion sensitive drinking.

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Poster

079. Alcohol's Effects on the Brain

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA R01AA13650
NIAAA F31 AA025518
NIH TR001106

Title: Frontal white matter integrity correlates with anterior cingulate cortex metabolites: An exploratory multimodal neuroimaging study of alcohol use disorder

Authors: *G. G. GRECCO[1], E. J. CHUMIN[2], M. DZEMIDZIC[3], H. CHENG[4], P. FINN[4], S. D. NEWMAN[4], K. K. YODER[1];

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[4]Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Multimodal imagining approaches are frequently used to address the neural changes associated with alcohol use disorder (AUD). Combined imaging techniques, such as magnetic resonance spectroscopy (MRS) and diffusion weighted imaging (DWI), may provide complementary and/or supplementary findings that may provide unique insights into tissue physiology and neuropathologies that may not be apparent with data from a single modality in isolation. However, few studies have taken advantage of this approach. In the present study, we examined the putative relationships between metabolites in the dorsal anterior cingulate cortex (dACC), a key region involved in addiction, and local WM integrity in tracts known to innervate the dACC. We hypothesized that there would be differences in the directionality of the relationships between young adult AUD and control (CON) subjects, potentially reflecting mechanisms underlying addictive processes. Sixteen AUD (22.2 ± 1.47 y.o.; 5 females) and fourteen CON (22.2 ± 3.42 y.o.; 8 females) underwent whole-brain DWI and structural magnetic resonance imaging (MRI) scans. MRS spectra were acquired from a voxel in the dACC (20 x 15 x 30 mm^3). Outcome measurements were relative metabolite concentrations in the dACC and scalar diffusion metrics in surrounding WM tracts. Linear regression was used to test for significant associations between metabolites and diffusion metrics for both the AUD and the CON group; Bonferroni correction for multiple comparisons was applied (*p* &lt 0.002). In the AUD group, there was a negative association between dACC glutamate and mean diffusivity in the right anterior corona radiata. In the controls, myo-inositol was positively correlated with axial diffusivity in the left anterior corona radiata. These are some of the first findings to indicate that the degree of local WM integrity may be related to metabolite concentrations in gray matter. Additionally, the data suggest that young adult AUD subjects may have distinct relationships between dACC metabolites and local WM integrity that may reflect either a predisposition to AUD, or the consequences of heavy drinking during the earliest stages of adult AUD. We suggest that future DWI studies should incorporate targeted investigations of MRS. Future multimodal imaging studies are warranted to better understand how DWI and MRS can inform the neuropathology underlying AUD.

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Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: National Basic Research Programno. 2015CB856400 of China
National Natural Science Foundation of China (Lin Lu, no. 81761128036)

Title: Disrupting smoking related memory reconsolidation attanuates craving and diminishes ACC reactivity to smoking cues

Authors: *X. LIN[1], J. DENG[3], L. SHI[2], L. LU[4];
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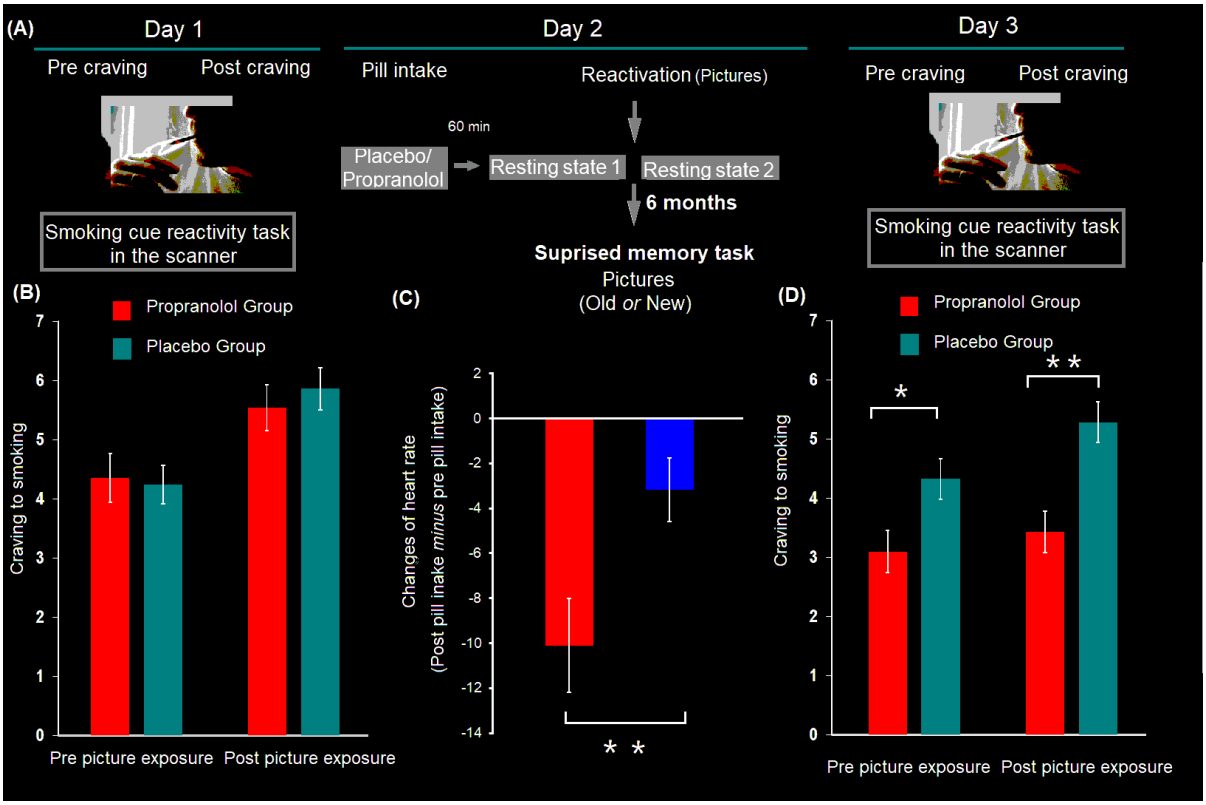
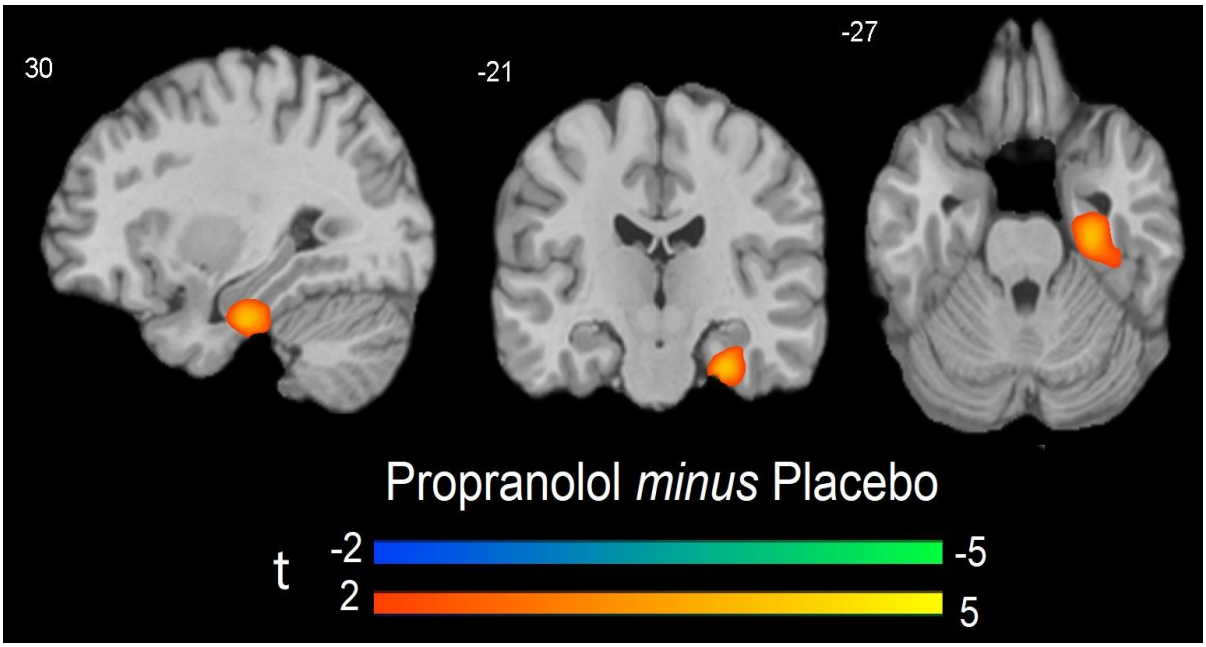
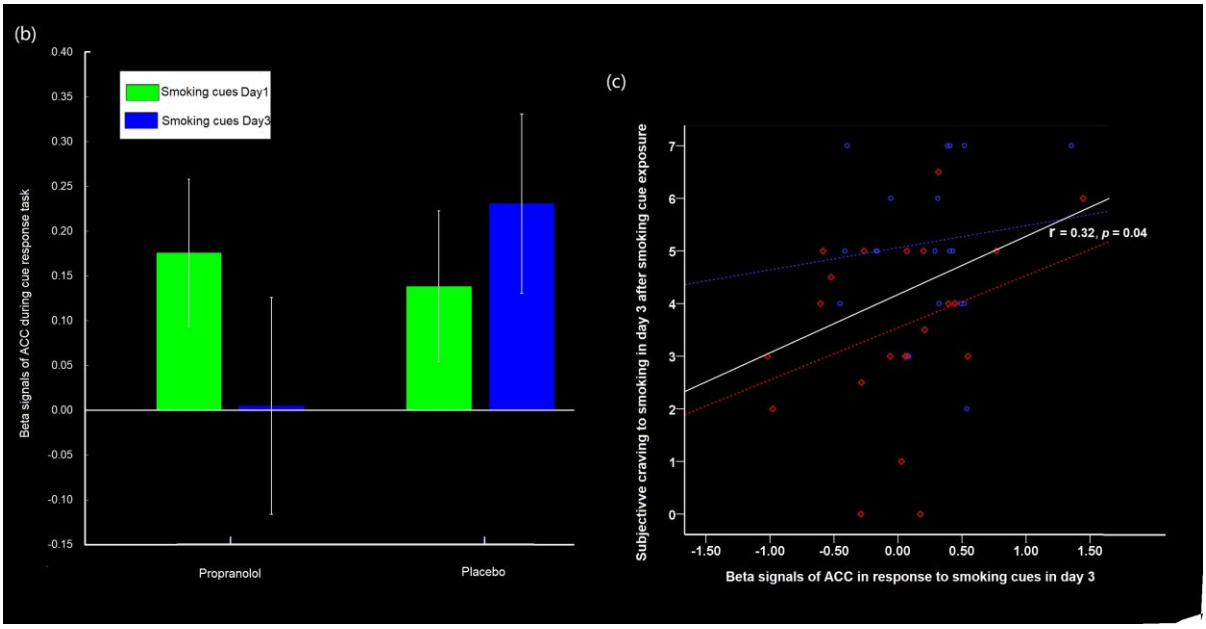
Abstract: From a memory perspective, nicotine addiction is established by a maladaptive association between environmental cues and smoking. We found that administration of the β-adrenergic receptor antagonist propranolol before memory reactivation decreased craving, with parallel diminished ACC reactivity to smoking cues, and resulted in memory impairment for smoking cues in smokers. This intervention not only cut off the association between cues and smoking, but also blocks smoking cues into long-term memory.







Disclosures: **X. Lin:** None. **J. Deng:** None. **L. Shi:** None. **L. Lu:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.02/X1

Topic: G.08. Drugs of Abuse and Addiction

Support: DePauw University

Title: Nicotine behavioral choice assays for larval zebrafish

Authors: *H. SCHNEIDER[1], A. PEARSON[2], S. KRAUSE[2], S. GOLDE[2], A. TUCKER[2], K. GARDNER[2], L. WHITE[2], D. ROSENE[2], D. HARRIS[2];
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Abstract: The ultimate goal of our projects is to identify potential new pharmacotherapeutics for the treatment of nicotine dependence, the number one cause of preventable diseases in the U.S. Zebrafish are a stepping stone towards developing better medicines that help people quit smoking. Larval zebrafish have advantages as experimental animals such as a small size, short developmental periods, availability of zebrafish mutants, targeted gene-knockout, and easy drug-delivery methods. Instead of measuring behavioral responses after bathing larval zebrafish in nicotine, behavioral choice tests could be a more effective way of discovering neural mechanisms and genes that are associated with or cause a higher predisposition of nicotine-dependence. Most recently, we have developed nicotine-seeking assays for larval zebrafish: a plus-maze, a gradient-maze and a rainbow maze test. While the plus maze and gradient maze tests were developed for nicotine-seeking assays, the rainbow maze test could be used for conditioned place preference tests.
Following an acclimation period in the maze, the behavior of larval zebrafish (6 to 8 dpf) was videotaped before and after the start of local delivery of nicotine into a single compartment of a maze. In subsequent video analyses using EthovisionXT (Noldus) the movement of larval zebrafish was tracked and variables such as cumulative time in a nicotine-containing compartment and frequency of entering a nicotine-containing compartment were measured. Results indicate that the assays can be used to distinguish nicotine-seeking from non-seeking larval zebrafish. Nicotine-seeking larval zebrafish occupy nicotine compartments for longer periods and enter the nicotine-containing compartments more frequently compared to non-seekers.
In summary, nicotine-seeking assays provide a method to distinguish nicotine-seeking from non-seeking larval zebrafish. The genetic analysis of nicotine-seeking larval zebrafish could lead to the identification of genes that facilitate nicotine-use behavior. Scaling of choice assays will yield a higher throughput and chemical screening of potential new pharmacotherapeutics for smoking cessation therapy.

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Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: NWO Vidi grant (016.Vidi.188.022)
Fulbright grant

Title: Chemogenetic inactivation of the insula decreases context-induced relapse to nicotine seeking after punishment-imposed abstinence

Authors: *R. J. HERMAN, Y. VAN MOURIK, D. SCHETTERS, T. J. DE VRIES, N. J. MARCHANT;
Amsterdam Univ. Med. Ctr., Amsterdam, Netherlands

Abstract: Smoking is one of the most preventable causes of death worldwide. In humans, attempts to quit smoking are often self-imposed due to the adverse effects of cigarettes. Relapse often occurs when people are exposed again to cues associated with smoking. Previous rodent studies of context-induced nicotine relapse do not adequately model the self-imposed component of human smoking abstinence. We developed a novel animal model to examine relapse to nicotine seeking after punishment-imposed abstinence. We trained rats (11 males and 12 females) to self-administer nicotine in one context. In a second context, one group of rats received nicotine infusion in combination with footshock for 50% of the reinforced nosepokes. Another group of rats received saline in the second context. We then tested the rats in extinction conditions in either the nicotine context (relapse), or the alternative context (Punishment, Saline). Both groups showed context-induced relapse in the nicotine-associated context compared to the alternate context. After the relapse test, we processed the brain tissue for Fos expression as a marker of neuronal activity. We found increased Fos in the anterior insula (AI) in the nicotine-paired context for both groups. We next sought to demonstrate a causal role for AI in context-induced relapse of nicotine seeking after punishment-imposed abstinence using chemogenetic inhibition of AI with hM4Di expression in AI under the CaMKIIa promoter. 7 male and 11 female rats were tested including inactive DREADD controls. We found that chemogenetic inactivation of the insula decreased context-induced relapse after punishment-imposed abstinence. We will present data from another experiment using the same manipulation in rats (11 males and 11 females) testing for context-induced reinstatement after extinction. These findings indicate that rats discriminate between contexts associated with nicotine and punishment of nicotine self-administration and these contexts retain the ability to promote (relapse) or suppress (abstinence) nicotine seeking after a period of abstinence. Furthermore, we show that the insula is an important neural substrate of context-induced relapse after punishment.

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Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.04/X3

Topic: G.08. Drugs of Abuse and Addiction

Support: DA041482

Title: Dynamic neural and circuit activity in the interpeduncular nucleus during nicotine withdrawal

Authors: *P. M. KLENOWSKI[1], R. ZHAO-SHEA[2], P. D. GARDNER[3], F. SUN[5], Y. LI[6], A. R. TAPPER[4];
[1]Univ. of Massachusetts, Worcester, MA; [2]Brudnick Neuropsychiatric Res. Inst., [3]Neurobio., [4]Univ. of Massachusetts Med. Sch., Worcester, MA; [5]Peking Univ. Sch. of Life Sci., Beijing, China; [6]Peking Univ., Beijing, China

Abstract: Previous studies have identified an important role for the interpeduncular nucleus (IPN) circuitry in somatic and affective symptoms of nicotine withdrawal. In particular, the medial habenula (mHb), which sends projections that co-release acetylcholine and glutamate to the IPN, is highly sensitive to nicotine due to dense expression of nicotinic acetylcholine receptors (nAChRs). Pharmacological and optogenetic manipulations of these projections is sufficient to induce both somatic and affective signs of nicotine withdrawal (Salas et al., 2009, Zhao-Shea et al., 2015). Additionally, recent work has uncovered a dopamine projection from the VTA which also releases corticotrophin releasing factor into the ventral portion of the IPN and is thought to play a role in the negative affective symptoms of nicotine withdrawal (Zhao-Shea et al., 2015). Despite these advances, little is known regarding the temporal dynamics of neuronal activity within this circuitry during onset and maintenance of nicotine withdrawal. Using in-vivo measurements of genetically encoded calcium sensors (GCaMP), we have identified significant alterations in GABAergic neuronal activity in various sub-regions of the IPN during mecamylamine induced, and spontaneous nicotine withdrawal. Additionally, we have utilized recently developed axonal specific GCaMP and genetically encoded GPCR-activation-based (GRAB) dopamine sensors, to dissect and map the contribution of mHb cholinergic/glutamatergic and VTA dopaminergic projections to various IPN sub-regions during the onset and maintenance of nicotine withdrawal. These studies will provide a greater understanding of the underlying temporal changes in neuronal activity that occur within this circuitry during nicotine withdrawal and may uncover additional mechanisms contributing to the high incidence of relapse in smokers.

Disclosures: **P.M. Klenowski:** None. **R. Zhao-Shea:** None. **P.D. Gardner:** None. **F. Sun:** None. **Y. Li:** None. **A.R. Tapper:** None.

Poster

080. Nicotine, Reward, and Dependence

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Program #/Poster #: 080.05/X4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant P50DA037844
NIH Grant R01AA024112

Title: Insular cortical GABAergic interneurons are critical for the cross-tolerance between nicotine and opiates

Authors: *G. C. LONEY[1], C. P. KING[2], C. E. BASS[3], P. MEYER[4];
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Abstract: Approximately 40% of opioid fatalities involve the misuse of legally prescribed opiate medication. Clinical studies demonstrate that smokers are less sensitive to the interoceptive stimulus properties of opiates displaying enhanced tolerance to both their aversive and reinforcing effects. As such, smokers are more likely to misuse prescribed opiates, potentially contributing to enhanced addition liability. The anterior insular cortex (aIC) is critically involved in processing and integrating external sensory cues and interoceptive awareness. Activation of ß2 nAChRs on GABAergic interneurons *in vitro* enhances GABAergic tone, ultimately inhibiting projection neurons of the aIC. Here, we tested the effect of local aIC infusions of nicotine (4 & 8 µg) on the aversive and reinforcing properties of morphine (5, 10, & 20 mg/kg) using conditioned taste avoidance (CTA) and conditioned place preference (CPP) paradigms, respectively. Nicotine reduced sensitivity to both the aversive and reinforcing effects of morphine evident by a significant rightward shift in the dose-response curves of both morphine-induced CTA and CPP. Next, we employed AAV viral constructs to drive Cre-mediated expression of the inhibitory DREADD hM4Di behind a GAD1 promotor in order to inhibit activity of GABA neurons within the aIC. Chemogenetic inactivation of aIC GABAergic interneurons blocked the ability for local aIC nicotine infusions to interfere with morphine induced CTA and CPP demonstrating the necessity of these GABAergic interneurons for these effects of nicotine. Finally, we tested the effects of nicotine on the within-session behavioral economics demand curve for remifentanil (RMF). Here the effort required to obtain RMF was held constant while the obtained dose of RMF was systematically decreased within session. We found that nicotine shifted the demand curve in a manner demonstrating that the dose at which responding for RMF began to decline was a higher dose following nicotine administration

compared to saline suggesting that rats were less sensitive to lower doses of RMF following nicotine treatment. In summary, we report, across three behavioral paradigms, that nicotine reduces sensitivity to the interoceptive stimulus properties of opiates and that this effect of nicotine is largely dependent on the ability for nicotine to activate GABAergic interneurons in the aIC.

Disclosures: G.C. Loney: None. **C.P. King:** None. **C.E. Bass:** None. **P. Meyer:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.06/X5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA T32 DA016176

Title: The effect of nicotine/cotinine on gut-brain axis communication via immune cell signaling through pro-inflammatory cytokine production

Authors: *J. LAKES, C. RICHARDS, M. FLYTHE;
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Abstract: There is mounting evidence suggesting the microbiome plays a key role in maintaining homeostasis in an organism through the bidirectional communication pathway known as the gut-brain axis. There are multiple routes through which communication may be occurring, however for my research purposes I have chosen to focus on the role of the immune system in this network of communication. Both the innate and adaptive arms of immunity collaborate to maintain homeostasis at the intestinal surface, or the host-microbiome interface. Not only does the immune system work directly with the microbiome, but it also exerts a bidirectional communication network with the central nervous system (CNS), in this way it acts as an intermediary between the gut and brain. Indirect effects of the gut microbiome on immune cells (dendritic cells/bone-marrow derived macrophages) can alter circulating levels of pro- and anti-inflammatory cytokines, which directly affect brain functioning/signaling. This is further altered in individuals who chronically use nicotine. The goal of this research was to elucidate the effect of nicotine on immune cell polarization in the context of the gut-brain axis.
I tested pro-inflammatory cytokine production (IFN-γ, IL-10, IL-12p70, IL-1β, IL-6, KC/GRO, TNF-α) of bone-marrow derived macrophages polarized to anti- or pro-inflammatory phenotypes untreated, or treated with a 1μM dose of either nicotine or cotinine. These macrophages were then treated with either metabolites and/or vesicles from the following members of the microbiome: *C. sticklandii, P. bryantii, B. fragilis*, or *A. muciniphila.* My results suggest that the presence of these bacterial products, regardless of species, exacerbates the pro-inflammatory

cytokine production across all cytokines tested. However, it also indicates that the presence of the alkaloid(s) including nicotine suppresses the pro-inflammatory response, which is further reduced when the bacterial products have also been treated with the alkaloid(s). These data suggest that chronic smoking/nicotine use may suppress the immune response to species within the microbiome, presumably altering the concentration of circulating pro- and anti-inflammatory cytokines and thus altering gut-brain axis communication through this pathway.

Disclosures: J. Lakes: None. **C. Richards:** None. **M. Flythe:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA038817
NIH Grant DA040047
NIH Grant DA046335

Title: Brain region specific single-molecule imaging show low dose nicotine drives structural changes in receptor assembly

Authors: *X. FU[1], F. H. MOONSCHI[1], A. M. FOX-LOE[3], A. J. AVELAR[4], B. J. HENDERSON[4], J. R. PAULY[2], C. RICHARDS[1];
[1]Chem., [2]Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY; [3]Chem., Slippery Rock Univ., Slippery Rock, PA; [4]Biomed. Sci., Joan C Edwards Sch. of Med. at Marshall Univ., Huntington, WV

Abstract: Nicotine, the primary addictive compound in tobacco, binds with high affinity to Neuronal nicotinic acetylcholine receptors (nAChRs). Exposure to nicotine can cause changes in expression, trafficking and stoichiometry of nAChRs, leading to modification in their function. α4β2 receptors, the most abundant nAChRs in the CNS, have two distinct stoichiometries, $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$. This study was conducted to study the effect of nicotine on the structural assembly of α4β2 receptors within an animal. We developed a single molecule technique to monitor changes in the structural assembly of α4β2 receptors in an animal in response to its physiological environment. The distribution of two isoforms of α4β2 receptors was quantified in the brain as a whole and individually in seven different brain regions from α4-GFP knock-in mice. The mice were given nicotine (0.7mg/kg/hr) or saline through osmotic pumps for 12 days. Nicotine administration was stopped to study effect of nicotine or animals were housed for another 7 and 21 days for nicotine withdrawal studies. We noticed that nicotine alters the expression of α4β2 receptors in the cortex and hippocampus, but not in the cerebellum,

hypothalamus, midbrain, striatum, and thalamus. The stoichiometry of α4β2 receptors in the cortex and hippocampus returns to the original population distribution after withdrawal. α4β2 receptors isolated from the cerebellum, midbrain, and thalamus, exhibit no significant changes in their distributions after nicotine withdrawal. This brain region specific single molecule study reveals selectivity of nicotine-induced upregulation in different brain regions correlated with changes in the distribution of receptor stoichiometry.

Disclosures: X. Fu: None. **F.H. Moonschi:** None. **A.M. Fox-Loe:** None. **A.J. Avelar:** None. **B.J. Henderson:** None. **J.R. Pauly:** None. **C. Richards:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.08/X7

Topic: G.08. Drugs of Abuse and Addiction

Title: Role of endocytic pathways in modulating nicotine-induced upregulation of α7 nicotinic acetylcholine receptor (nAChR) downstream of G protein interactions in Xenopus oocytes

Authors: *J. PANCHAL[1], M. ISLAM[3], K. DEBOEUF[4], J. B. ANDERSON[5], J. FARLEY[1], I. MCFATRIDGE[2];
[2]Neurosci., [1]Indiana Univ., Bloomington, IN; [3]Neurosci., [4]Psychology and Neurosci., [5]Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN

Abstract: Nicotinic acetylcholine receptors (nAChRs), member of cys-loop receptors, mediate many cellular and behavioral functions throughout the nervous system. α7 nAChR, the second most abundant nAChR expressed in brain, has been shown to play an important role in nicotine addiction. Previously, upregulation of α7 nAChRs by nicotine has been reported in several studies, though the mechanisms remain unclear. We have shown ~2-fold functional and numerical upregulation of α7 Rs in *Xenopus* oocytes following 100 μM nicotine treatment overnight and 7 hr hourly washout. Further, we have found that the nic-upregulation of α7 nAChR was intracellular calcium-dependant but independent of extracellular calcium. In various types of cells, G protein signaling regulates α7 nAChR function via modulation of intracellular calcium levels. α7 nAChR, in the transmembrane (TM) 3-4 cytosolic loop, contains RMKR sequence as a G-protein binding cluster (GPBC). Here, we demonstrate a role for α7 nAChR/$G\alpha_q$ protein interaction in the activation of PLC β pathway leading to prolonged intracellular calcium release that, in turn, inhibits endocytic pathways to cause upregulation of α7 nAChRs by nicotine. Mutation at GPBC, preventing interaction of endogenous $G\alpha_q/G\beta\gamma$ with GPBC and disrupting $G\alpha_q$-PLC-IP_3-Ca^{2+} release, abolished nic-upregulation of α7 Rs. Furthermore, when $G\alpha_q$ was exogenously co-expressed with wt α7 Rs, we saw ~2X upregulation, but when dominant negative $G\alpha_q$ was co-expressed, a significant attenuation of nic-upregulation of wt α7

Rs was observed. We also found that a calcineurin inhibitor cyclosporine A produced 2X-upregulation of wt α7 Rs and occluded nic-upregulation. Endocytic inhibitors produced ~2X upregulation of both wt and mutant α7 Rs and occluded nic-upregulation. Brefeldin A (BFA, inhibitor of protein transport from ER to Golgi) failed to affect nic-upregulation. Interestingly, prolonged exposure to the membrane-permeable competitive antagonist methyllycaconitine (MLA) upregulated α7 Rs in a calcium-independent manner, and inhibition of MLA-induced upregulation by BFA supported a chaperone-role for MLA. Our results suggest that nicotine and MLA upregulate α7 Rs by different mechanisms. Serine-threonine kinase and tyrosine kinase-phosphorylation events have inhibitory effects on forward trafficking of α7 Rs. Nic-upregulation was unaffected when serine-365 or tyrosine-442 (from TM3-4 loop) was mutated, further supporting the hypothesis that nic-upregulation of α7 arises from sustained GPCR-Ca^{2+}-signaling by α7 Rs, inhibiting basal/constitutive endocytosis of α7 Rs.

Disclosures: **J. Panchal:** None. **M. Islam:** None. **K. Deboeuf:** None. **J.B. Anderson:** None. **J. Farley:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.09/X8

Topic: G.08. Drugs of Abuse and Addiction

Support: National Institute of Drug Abuse DA021274
National Institute of Drug Abuse DA033613
National Institute of Drug Abuse HHSN271201600057C

Title: Female rats display greater neuronal activation in the interpeduncular nucleus during nicotine withdrawal than males

Authors: *F. MATOS[1], P. CORREA[1], V. CORREA[2], L. M. CARCOBA[1], S. D. INIGUEZ[1], A. R. ZAVALA[3], L. E. O'DELL[1];
[1]Psychology Dept., The Univ. of Texas At El Paso, El Paso, TX; [2]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Psychology Dept., California State Univ., Long Beach, CA

Abstract: Pre-clinical studies in male rodents has established that the medial habenula-interpeduncular nucleus (MHb-IPN) pathway plays a role in the expression of the negative affective states produced by nicotine withdrawal. Previous work has also revealed that there are sex differences in the behavioral effects of nicotine, with female rats displaying greater anxiety-like behavior during withdrawal as compared to males. Despite profound behavioral differences, the role of the MHb-IPN pathway in modulating sex differences in the behavioral effects of nicotine withdrawal remains unexplored. As a first step in addressing this issue, the present study

compared neuronal activation via cFos expression in the IPN of female and male rats during nicotine withdrawal. Briefly, adult male and female rats received an osmotic pump that delivered nicotine continuously (3.2 mg/kg/day) and controls received sham surgery. Fourteen days later, the rats received an injection of the nicotinic receptor antagonist, mecamylamine (3.0 mg/kg, SC) to precipitate withdrawal. Ninety minutes later, the rats were euthanized and perfused with 4% formaldehyde. The brains were sliced and processed for immunofluorescence using an anti-cFos antibody (ab190289) and a secondary antibody (Alexa Fluor 488, A11008) to visualize the neurons and facilitate cell counting. The results revealed that control female and male rats did not display neuronal activation in the IPN. However, during withdrawal, females displayed approximately 2-fold higher levels of neuronal activation in the IPN than males. These results suggest that the IPN modulates sex differences in nicotine withdrawal. Ongoing studies are examining cFos activation in a preparation involving double labeling with glutamate decarboxylase (GAD) in order to examine whether the present results are due to activation of GABAergic neurons in the IPN.

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Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.10/X9

Topic: G.08. Drugs of Abuse and Addiction

Support: R00 DA036569
R03 DA045881
R21 DA044479
Arizona Alzheimer's Consortium

Title: Ovarian hormones mediate acquisition of nicotine self-administration and accumbens glutamatergic plasticity

Authors: P. F. OVERBY, J. M. LEYRER-JACKSON, J. PIÑA, H. ULANGKAYA, *M. D. NAMBA, H. A. BIMONTE-NELSON, C. D. GIPSON;
Dept. of Psychology, Arizona State Univ., Tempe, AZ

Abstract: Nicotine addiction in women remains a significant public health liability. Women report greater craving during certain phases of the menstrual cycle, and as such, pharmacotherapies for smoking may be less efficacious in women compared to men, possibly due to interactions with ovarian hormones. Mechanistically, 17-β-estradiol (E2) receptors are located on GABAergic medium spiny neurons (MSNs) within the nucleus accumbens core

(NAcore). Synapses on NAcore MSNs undergo rapid, transient plasticity during nicotine seeking due to increased extracellular glutamate during nicotine seeking. We hypothesized that ovarian hormones play an important role in the acquisition of nicotine self-administration as well as NAcore glutamatergic plasticity. Female Sprague-Dawley rats were left intact or ovariectomized (OVX), followed by intravenous jugular catheter implantation. Animals then underwent nicotine self-administration, where the active lever yielded one infusion (0.02 mg/kg/infusion, i.v.) paired with a compound stimulus (lights + tone). A subset of OVX females received E2 supplementation for the last 4 days of self-administration. Animals were then sacrificed for electrophysiological recordings. OVX females acquired nicotine self-administration at a slower rate compared to intact females ($p<0.05$). E2-treatment increased self-administration to levels similar to intact females. Additionally, deprivation of ovarian hormones due to OVX potentiated NAcore MSNs following nicotine self-administration, which was reversed by E2-treatment in OVX females ($p<0.05$). These results suggest that ovarian hormones mediate nicotine reinforcement. As well, these results indicate that following nicotine self-administration in intact females, NAcore synapses rest in a depotentiated state, which may increase nicotine use vulnerability. Finally, cessation of ovarian hormones induces metaplasticity in NAcore synapses, which is reversed by E2-treatment. Taken together, these studies reveal control of ovarian hormones on nicotine addiction and underlying NAcore glutamatergic plasticity.

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Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.11/DP11/X10

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA044479
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NIH Grant DA045881

Title: Chemogenetic inhibition of accumbens cholinergic interneurons inhibits cue-induced nicotine seeking

Authors: J. M. LEYRER[1], M. HOLTER[2], M. BRICKNER[3], J. NEWBERN[2], P. F. OVERBY[1], M. F. OLIVE[4], ***C. D. GIPSON**[1];
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Abstract: Nicotine is the primary addictive substance in tobacco and is widely abused. Due to the recent surge in e-cigarette use especially adolescents and young adults, it is essential to understand underlying neurophysiological mechanisms of nicotine addiction. While previous studies have shown that increased glutamate release from prelimbic afferents targeting the nucleus accumbens core (NAcore) contributes to cue-induced reinstatement, the role cholinergic interneurons (ChIs) within the nucleus accumbens in mediating nicotine-seeking behavior is unknown. The principle hypothesis for the conducted studies is that cue-induced glutamate release from prefrontal cortical projections into the NAcore activates ChIs, and this induces acetylcholine (ACh) release through activation of nicotinic acetylcholine receptors (nAChRs). We hypothesize that ACh released from ChIs is a feedforward mechanism that promotes additional glutamate release from prelimbic afferents, which exacerbates relapse of nicotine seeking. In this way, ChIs modulate glutamatergic signaling, transitioning drug craving to drug seeking. Using choline acetyltransferase (ChAT)-Cre transgenic rats, ChIs were bi-directionally manipulated prior to cue-induced reinstatement using chemogenetics. Prior to self-administration, cannulae were placed into the NAcore, and Cre-dependent inhibitory, excitatory and control DREADD vectors packaged in AAVs were then bilaterally infused into the NAcore allowing for chemogenetic control of NAcore ChIs. Rats underwent nicotine self-administration (0.02 mg/kg/infusion), in which an infusion was paired with a compound stimulus (discrete lights + tone) for 10 sessions. Following nicotine self-administration, rats were placed into daily extinction sessions, where no nicotine or cues were delivered upon active lever presses, for a minimum of 14 days. Prior to cue-induced reinstatement, intra-NAcore clozapine-N-oxide (CNO) was administered. Following reinstatement, whole-cell electrophysiology was conducted from medium spiny neurons (MSNs) within the NAcore to identify changes in synaptic plasticity (measured via AMPA/NMDA ratio). Results show that chemogenetic inhibition of ChIs inhibits cue-induced reinstatement. Ongoing studies are identifying the effects of chemogenetic activation of ChIs on cue-induced reinstatement and the changes in MSN synaptic plasticity. The results of the current study will uncover the role of ChIs in nicotine relapse and may provide mechanisms beneficial for therapeutic development for treatment of nicotine addiction.

Disclosures: **J.M. Leyrer:** None. **C.D. Gipson:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: G.02. Motivation

Support: NIH Grant R01-DA042889
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HHMI Gilliam fellowship

NSF
Wayne and Gladys Valley Foundation

Title: Identification of a brainstem-midbrain circuitry underlying nicotine reward and aversion

Authors: C. LIU[1], A. TOSE[1], J. X. DU[2], Y. ZHU[1], J. W. DE JONG[1], K. T. BEIER[3], *S. LAMMEL[1];
[1]UC Berkeley, Berkeley, CA; [2]UCSD, San Diego, CA; [3]UC Irvine, Irvine, CA

Abstract: Nicotine and other addictive drugs are said to hijack the brain's reward pathways. Decades of studies have revealed dopamine (DA) neurons in the ventral tegmental area (VTA) as key players in mediating nicotine reward. Unlike most other addictive drugs, nicotine induces aversion at high doses but the underlying mechanism is not well understood. The lateral dorsal tegmentum (LDT) is a brainstem structure required for VTA DA burst firing and nicotine self-administration. It contains excitatory and inhibitory neurons that project to VTA. The interpeduncular nucleus (IPN), located immediately ventromedially to the VTA, projects to the LDT and has been implicated in nicotine aversion. We hypothesized that separate cell populations within this circuit have distinct roles for mediating nicotine's opposing effects. To dissect the circuitry underlying nicotine reward and aversion, we employed retrograde tracing, optogenetics, fiber photometry, electrophysiology and behavioral experiments. We first performed a detailed anatomical and functional characterization of LDT inputs and outputs. We found that the majority of VTA-projecting LDT neurons (LDT→VTA) are glutamatergic (~60% VGlut2), whereas a smaller number of cells are GABAergic (~30% GAD2). Only ~5% of all LDT neurons co-express both VGlut2 and GAD2 transcripts. Additional viral tracing and electrophysiology experiments suggest that LDT_{GABA} and LDT_{Glut} neurons differ in their inputs as well as downstream projections, including the IPN and VTA. By performing *in vivo* optogenetic manipulations, we show that LDT_{Glut} and LDT_{GABA} neurons trigger reward- and aversion-associated behaviors through activation or inhibition, respectively, of subpopulations in the VTA and/or IPN. Next, we investigated the effects of rewarding and aversive doses of intravenously-administered nicotine on distinct circuit elements of the LDT-midbrain system. *In vivo* fiber photometry recordings reveal that VTA DA neurons are strongly activated by a rewarding lower dose of nicotine, but are inhibited by an aversive higher dose. In addition, LDT_{GABA}→VTA terminals are strongly activated by an aversive dose of nicotine, but this activation is weaker in response to a rewarding dose of nicotine, suggesting that LDT_{GABA}-mediated inhibition of VTA DA neurons may contribute to nicotine's aversive effects. We are currently investigating the effects of rewarding and aversive doses of nicotine on the LDT_{GABA}→IPN and LDT_{Glut}→VTA pathways. A detailed understanding of the circuitry between brainstem and midbrain subregions may contribute to the development of targeted pharmaceutical treatments to combat nicotine addiction.

Disclosures: S. Lammel: None. **C. Liu:** None. **A. Tose:** None. **J.X. Du:** None. **Y. Zhu:** None. **J.W. de Jong:** None. **K.T. Beier:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.13/X12

Topic: G.08. Drugs of Abuse and Addiction

Title: Physiological investigation of reward responsiveness between nicotine users and nonusers

Authors: *C. A. LOVE, A. PALMISANO, O. OKIFO, M. PADUA, R. LIVOTI, K. JENKINS, M. MOURMOURAS, K. MILLER, A. PURINS, G. CIAMBRIELLO, R. S. ASTUR;
Univ. of Connecticut, Storrs, CT

Abstract: Repeated exposure to nicotine has been associated with increased incentive salience to neutral stimuli, possibly through sensitization of dopaminergic reward circuitry. This in turn strengthens nicotine users' appetitive behavior. Despite both neuroimaging and behavioral studies on the topic, it is still unclear if users are hyperreactive to drug rewards and thus hyporeactive to non-drug stimuli, or if their salience to all rewards is enhanced regardless of the drug association. The present study examined the role of nicotine use in characterization of motivational valence in both nicotine and non-nicotine reward cues. Participants were either active nicotine users or nonusers, as measured by a battery of questionnaires. Participants of both groups were presented with a series of standardized positive, negative, or neutral valenced non-nicotine images from the International Affective Picture System (IAPS) and standardized nicotine images from the International Smoking Image Series (ISIS). They were asked to rate the valence and arousal of each image while physiological skin conductance responses (SCRs) were collected. We hypothesized that nicotine users would rate nicotine-related images higher in both subjective valence and arousal and show a greater SCR to these stimuli than the nonusers. Nicotine users (n = 78) rated nicotine images as significantly more pleasurable as well as more arousing than did the nonusers (n = 79*)*. Additionally, nicotine users' SCRs were significantly greater in response to nicotine images than those of nonusers. Nicotine users showed no significant differences from nonusers in subjective valence or arousal ratings between affectively-positive, affectively-negative, or neutral images, and there were no between-group differences in SCRs to these images. Within the nicotine user group, specifically, we hypothesized higher ratings of nicotine stimuli than neutral and affectively-negative stimuli; however, given discrepancies in the literature, we made no predictions regarding the direction of responses assigned to affectively-positive stimuli. In line with the hypothesis, nicotine users rated nicotine-related images significantly more pleasurable than negatively valenced images and more arousing than neutral images. Within the nicotine user group, positive images were rated significantly more pleasurable and arousing than nicotine images, despite greater SCRs to nicotine rather than affectively-positive images. Present findings suggest that nicotine users

attribute enhanced incentive salience to nicotine stimuli compared to nonusers, but the groups do not differ in reactivity to non-nicotine rewards.

Disclosures: **C.A. Love:** None. **A. Palmisano:** None. **O. Okifo:** None. **M. Padua:** None. **R. LiVoti:** None. **K. Jenkins:** None. **M. Mourmouras:** None. **K. Miller:** None. **A. Purins:** None. **G. Ciambriello:** None. **R.S. Astur:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.14/X13

Topic: G.08. Drugs of Abuse and Addiction

Title: Association between brain alterations and neuropsychological scales in the patients with nicotine addicting

Authors: *Y. CHUANG[1], M. HO[2,3], J. WENG[1,4];
[1]Chang Gung Univ., Taoyuan City, Taiwan; [2]Dept. of Psychology, Chung Shan Med. Univ., Taichung, Taiwan; [3]Clin. Psychological Room,, Chung Shan Med. Univ. Hosp., Taichung, Taiwan; [4]Dept. of Psychiatry, Chang Gung Mem. Hosp., Taoyuan City, Taiwan

Abstract: Different addictive substances affect the brain and behavior in different ways and determine the duration of their effects on the body. Nicotine is a stimulant with a short half-life, which means that smokers need to intake tobacco to maintain the carnival effect in a short period of time. We tried to find the brain volume changes in specific brain regions affected by tobacco addiction. The neuropsychological scales were examined, including Fagerstrom Test for Nicotine Dependence (FTND), which shows the dependence of the smokers on smoking addiction, and Drug Use Disorders Identification Test (DUDIT), which presents the treatment motivation of the smokers. High-resolution T1-weighted images of seventy smokers' brain were acquired using a 3T imaging system (GE Medical Systems, Waukesha, WI). In voxel-based morphometry (VBM) analysis, all anatomic images were preprocessed using Statistical Parametric Mapping 8 (SPM8, Department of Cognitive Neurology, London, UK) with Voxel-Based Morphometry 8 (VBM8, University of Jena, Department of Psychiatry, Jena, Germany) toolbox. Every raw data were first normalized to International Consortium for Brain Mapping (ICBM) templates, East Asian Brain. The multiple regression was performed to obtain the association between brain volume of gray/white matter and FTND/DUDIT in each smoker. To avoid the original difference of each brain, age, education years, and whole brain volume were excluded as covariates. All of the results were statistically significant (corrected $p < 0.05$). We observed the correlation between FTND scale and brain volume of gray/ white matter in the limbic lobe/ sub-lobar. The higher the score of the FTND scale, the smaller the brain volume of limbic lobe, which could be indirectly related to one's long-term memory and negative emotions. Additionally, the positive correlation

between DUDIT scales and brain volume of gray/ white matter was found in the brainstem/ frontal lobe. The higher the score of the DUDIT scale, the larger the brain volume of frontal lobe, which is associated with the ability to regulate one's own emotions and impulses. It could let smokers value the issue of nicotine addicting, and encourage to find out the solution for reducing the daily tobacco intake as soon as possible.

Disclosures: **Y. Chuang:** None. **M. Ho:** None. **J. Weng:** None.

Poster

080. Nicotine, Reward, and Dependence

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Program #/Poster #: 080.15/X14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA039658 to CDF

Title: To vape or not: Nicotine E-cigarette vapor inhalation in a rodent model

Authors: *V. LALLAI[1], C. D. FOWLER[2];
[1]UCI, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: E-cigarettes, which deliver vaporized nicotine, have dramatically risen in popularity in recent years and are now regularly being used by millions, despite many unanswered questions about safety, efficacy in reducing dependence, and overall impact on public health. Other factors, such as sex and age, also play an important role in determining behavioral and neurochemical responses to drugs of abuse. Given this, we sought to develop a protocol for vaporized nicotine exposure in rats to provide a basis to better understand the differing effects of nicotine exposure routes on brain function. Vaporized nicotine inhalation was compared to intravenous nicotine self-administration, the current procedure most commonly accepted as having high translational relevance to the patterns of nicotine intake found in humans. In the current studies, male and female Wistar rats were separated into three groups: (1) nicotine intravenous self-administration, (2) passive vapor nicotine exposure, and (3) nicotine vapor self-administration.Allanimals were subjected to blood sample collection after sessions to assess levels of cotinine, the main metabolite of nicotine, through immunological assay (ELISA).After drug sessions, subjects were also examined in an open-field to assess nicotine-mediated differences in locomotor activity. We found that exposure to vapor induced similar levels of cotinine as intravenous nicotine self-administration in male rats, indicating that the breathing patterns with vapor exposure led to similar levels of nicotine intake. Interesting, a differential effect was found in the female rats, in which the same conditions of vapor exposure led to decreased cotinine levels for vapor compared to intravenous self-administration. Differences in nicotine-mediated locomotion provide further support of the level of nicotine intake with both methods. Together, these data validate a protocol

for vapor nicotine self-administration in rats, with high relevance to established techniques in the field, and the findings further highlight important sex differences in nicotine consumption based on the route of intake. With this foundation, ongoing studies are utilizing this approach to better understand the impact of e-cigarettes on health and processes underlying nicotine dependence.

Disclosures: V. Lallai: None. **C.D. Fowler:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01-DA034389

Title: Investigating the tobacco constituent menthol as an interoceptive stimulus or a modulator of the nicotine stimulus in rats

Authors: *Y. W. HUYNH, A. E. MORAN, A. RAIMONDI, A. FINKNER, R. A. BEVINS; Psychology, Univ. of Nebraska Lincoln, Lincoln, NE

Abstract: Menthol is a widely used tobacco constituent. Preclinical research with rat nicotine self-administration suggests that menthol enhances nicotine reinforcement. If injected menthol alters the reinforcing effects of nicotine in rats, we hypothesized that injected menthol may also have stimulus effects (Experiment 1) and/or modulate the stimulus effects of nicotine (Experiment 2). In Experiment 1, we investigated whether menthol alone set the occasion for when a discrete conditioned stimulus (CS, cue light illumination) was paired with sucrose access. Sprague-Dawley rats (10M, 10F) received intermixed positive and negative training days, which consisted of eight 15-sec CS trials across 20 min. On positive days, rats were pretreated with menthol (0.0183 or 5 mg/kg) and sucrose access was available in the dipper after each CS trial. On negative days, rats were pretreated with the vehicle (50% DMSO:H2O, v/v) and given eight CS trials without sucrose. CS-specific goal-tracking was used as the primary dependent measure. After 16 menthol and 16 vehicle days, rats that received 0.0183 mg/kg menthol were shifted to 15 mg/kg menthol. Next, the injection-to-placement-interval switched from 5 to 15 min for an additional 32 days. Finally, a subset of menthol trained rats (n=10) received nicotine training for 20 positive (0.4 mg/kg nicotine) and 20 negative (saline) days. In Experiment 2, 57 naïve rats (28M, 29F) were assigned to one of six groups. Each group received a unique dose combination of menthol (0, 1, or 5 mg/kg) and nicotine (0.1 or 0.4 mg/kg) on positive days; all groups received a vehicle and saline injection on negative days. After 40 training days, all rats underwent substitution testing to create a dose-response curve for 30 menthol-nicotine combinations. For test days, rats received one non-reinforced light cue in a 4-min session. In

Experiment 1, rats never acquired a discrimination using menthol alone; however, the nicotine-trained subset did acquire the discrimination. In Experiment 2, there was only a significant effect of nicotine dose for both training and testing sessions. These data suggest that injected menthol does not serve as an occasion setter stimulus or modulate the stimulus effects of nicotine at doses that alter nicotine self-administration.

Disclosures: Y.W. Huynh: None. **A.E. Moran:** None. **A. Raimondi:** None. **A. Finkner:** None. **R.A. Bevins:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.17/X16

Topic: G.08. Drugs of Abuse and Addiction

Support: FAPERJ
CNPq
CAPES
UERJ

Title: Nicotine exposure in Swiss adolescent mice: Does subsequent treatment with varenicline modify anxiety-like behavior and the pattern of nicotine consumption during re-exposure at adulthood?

Authors: *A. C. MANHAES, **V. H. S. D. PINHEIRO**, B. M. LOTUFO, F. U. BRAGA, A. T. M. A. CYPRIANO, C. C. FILGUEIRAS, Y. ABREU-VILLAÇA;
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Abstract: Nicotine is the main pharmacologically active substance in cigarettes responsible for addiction. It has been shown that teenagers that are exposed to nicotine have increased risks of acquiring long lasting addiction. Strategies to facilitate the interruption of nicotine use have been developed, but little is known about the short- and long-term effects of nicotine exposure during adolescence and even less of the available treatments for addiction reversal. The objective of this work was to analyze behavior in the Elevated Plus Maze (EPM) and Open Field (OF) of mice exposed to nicotine during adolescence and then treated with varenicline. We also analyzed the preference for nicotine in the two-bottle choice test. From postnatal day 30 (PN30) to PN45, 120 Swiss mice were exposed to a 2% saccharine solution containing 50µg/mL of nicotine (NIC) or to a 2% saccharine solution (SAC) or filtered water (H2O). From PN46 to PN55, animals were treated orally (gavage) with 0.1mg/Kg varenicline (VAR) or filtered water (CTRL). The following experimental groups were tested (exposure-treatment): 1) SAC-VEH; 2) SAC-VAR; 3) NIC-VEH; 4) NIC-VAR; 5) H2O-VEH 6) H2O-VAR. Animals were tested in the EPM at

PN54.At PN56, animals started the two-bottle choice (TBC), where the animals had two bottles freely available to drink from in their home cages: One with NIC and the other with SAC. The concentration of NIC during the first 4 days of TBC was maintained at 50mg/mL NIC. After a withdrawal period (PN60-62), the two-bottle choice test resumed (PN63 onwards): Every two days (for six days), the nicotine concentration was reduced (fading period): 50, 10, and 2 μg/mL. A multivariate ANOVA failed to find differences between groups regarding anxiety levels in the EPM, but indicated that locomotion was increased by VAR (p 0.007). In the OF, locomotion was increased in the periphery in NIC-VAR animals when compared to H2O-VAR ones (p = 0.021). Regarding TBC, NIC-exposed animals had higher preference for nicotine during the initial 4 TBC days (p=0.018). The aforementioned results indicate that anxiety-like behavior is not affected by varenicline by the end of the treatment period nor does this drug affects nicotine preference at adulthood.

Disclosures: **A.C. Manhaes:** None. **V.H.S.D. Pinheiro:** None. **B.M. Lotufo:** None. **F.U. Braga:** None. **A.T.M.A. Cypriano:** None. **C.C. Filgueiras:** None. **Y. Abreu-Villaça:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.18/X17

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA024112

Title: Nicotine enhances reinstatement of ethanol-seeking induced by a contextually controlled discriminative-stimulus

Authors: *H. ANGELYN[1], P. MEYER[2];
[2]Psychology, [1]Univ. At Buffalo, Buffalo, NY

Abstract: Nicotine is a powerful reinforcement-enhancer, and thus likely promotes alcohol (ethanol) self-administration by facilitating the response to stimuli that coincide with ethanol delivery. However, it is not clear whether nicotine also alters the response to stimuli that modulate, as opposed to reinforce, ethanol self-administration by signaling ethanol *availability*. To investigate this, we trained rats in a paradigm wherein presentations of a discriminative stimulus (S^D; a light) signaled time periods when completion of an operant would result in access to ethanol, and then assessed the effect of nicotine on the ability of the S^D to reinstate ethanol-seeking. We found that acute and chronic nicotine enhanced S^D –induced reinstatement. In real-life situations, however, contexts often inform relationships between discriminative stimuli and response-outcome associations, and thereby control whether discriminative stimuli induce behaviors. For example, although drinkers may inhibit their responses to alcohol-associated

stimuli in an alcohol-free treatment context, drinkers may relapse to alcohol use when they encounter the same stimuli in their normal drinking context. Therefore, we also tested whether nicotine facilitates S^D-induced reinstatement in a self-administration context after learning the S^D is not informative in an extinction context. Male and female Long-Evans rats underwent ethanol self-administration, extinction and reinstatement in an ABA paradigm, throughout which they received nicotine or saline injections during self-administration and/or reinstatement in a 2 x 2 design. During self-administration (context A), presentations of the S^D signaled 'drug available' periods when nosepokes into an active port would result in access to a sipper-bottle containing ethanol (15% *v/v*) for 30-s. Presentations of the S^D were alternated within session with presentations of the S^{delta} which signaled 'drug unavailable' periods when nosepokes would not be reinforced. During extinction (context B), presentations of the S^D and S^{delta} occurred as in self-administration; however, nosepokes were not reinforced. During reinstatement (context A), we measured the ability of presentations of the S^D and S^{delta} to induce ethanol-seeking in the absence of reinforcement. Preliminary findings suggest that, while nosepoking was inhibited in the extinction context, nicotine enhanced ethanol seeking induced by presentations of the S^D, and not the S^{delta}, in the self-administration context. Thus, we demonstrate a novel mechanism by which nicotine enhances the motivation for ethanol by affecting a response-independent, modulatory stimulus.

Disclosures: H. Angelyn: None. **P. Meyer:** None.

Poster

080. Nicotine, Reward, and Dependence

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 080.19/X18

Topic: G.08. Drugs of Abuse and Addiction

Support: BBRF Grant #24994
UTEP School of Pharmacy

Title: Investigating the effects of nicotine vapor exposure on impulsive choice

Authors: R. J. FLORES[1], P. GINER[1], T. G. MIRAMONTES[1], ***I. A. MENDEZ**[2];
[1]Psychology, [2]Sch. of Pharm., Univ. of Texas at El Paso, El Paso, TX

Abstract: *Background:* Studies with humans have shown that nicotine cigarette smokers exhibit increased impulsive choice relative to non-smokers. Pre-clinical studies investigating the effects of nicotine injections on impulsive choice in rodents have also demonstrated that nicotine increases impulsive choice. Surprisingly, research investigating the effects of nicotine vapor exposure on impulsivity has not been conducted. The goal of this study was to investigate the effects of nicotine vapor exposure on impulsive decision-making, using the delay discounting

task. *Methods:* Twenty-four adult male rats trained in the delay discounting task, in which rats are allowed to choose between a small immediate food reward or a larger food reward with delayed deliveries. After training in the discounting task for 24 days, rats were passively exposed to vapor containing either 0, 12, or 24 mg/ml of nicotine for 10 days, using a novel nicotine vapor exposure system. Blood samples were collected on selected exposure days and ELISA was used to assess blood serum cotinine levels. Following nicotine vapor exposure, rats were retrained until stable responding was achieved in the discounting task and the effects of nicotine vapor on choice preference in the discounting task was assessed. *Results:* Analysis of blood serum levels revealed cotinine in the 12 and 24 mg/ml groups, but not the 0 mg/ml group. Preliminary data from the discounting task suggests that nicotine vapor exposure shifts choice preference towards the small immediate reward (increases impulsive choice), when compared to controls. *Conclusions:* Initial findings suggest that repeated exposure to nicotine vapor can cause persistent changes in decision making. Additional research on the effects of nicotine vapor exposure on the brain and behavior is necessary, as electronic cigarette use has now surpassed traditional cigarette use in adolescents.

Disclosures: R.J. Flores: None. **P. Giner:** None. **T.G. Miramontes:** None. **I.A. Mendez:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.01/X19

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH104259
NSF Neuronex Grant 1707287
Whitehall Foundation Research Grant
UC Mexus Postdoctoral Fellowship
Harvey L. Karp Discovery Award

Title: Population dynamics of cortical activity in retrosplenial cortex during spatial decisions in virtual environments

Authors: *L. M. FRANCO[1], M. J. GOARD[1,2,3];
[1]Neurosci. Res. Inst., [2]Dept. of Molecular, Cell. and Developmental Biol., [3]Dept. of Psychological and Brain Sci., Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: Neurons undergo constant synaptic turnover during memory formation and maintenance. However, it is not clear how dynamic neural networks stably store learned associations during performance of a stable behavior. In order to better understand network

dynamics underlying memory, it is necessary to chronically monitor large-scale neuronal activity during performance of a learned behavior. To address this, we have developed a context-based virtual navigation task in which head-fixed mice must select a turn based on the spatial context. After training, we imaged population neural activity in superficial cortex using widefield and two-photon microscopy. We observe strong task-modulated activity in retrosplenial cortex. Although retrosplenial cortex is known to be important for spatial navigation, it is not known how retrosplenial neurons respond during spatial decision making. We find that retrosplenial neurons exhibit task-locked responses, organized in time, forming a sequence of neural activation. Moreover, individual neurons and populations of neurons distinguish between spatial contexts, location in the maze, and turning decisions. These spatial representations change dynamically across days, yet higher order patterns are maintained. Together, our results show that retrosplenial cortex multiplexes visual, spatial, and motor representations during virtual navigation, representing task information in a stable manner at the population level, even as individual neurons exhibit variable responses across days.

Disclosures: L.M. Franco: None. **M.J. Goard:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.02/X20

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant T90DA043219

Title: Up down alternations in a self-balancing spiking network

Authors: *J. GORNET[1], D. LEVENSTEIN[2], G. BUZSAKI[3], J. M. RINZEL[4];
[1]Washington Univ. In St. Louis, St Louis, MO; [2]Ctr. for Neural Sci., NYU, New York, NY; [3]New York University, Sch. of Med., New York, NY; [4]Ctr. Neural Sci., New York Univ. Ctr. for Neural Sci., New York, NY

Abstract: During NREM sleep, neural populations in the neocortex alternate between DOWN states and UP states of low rate asynchronous spiking, during which neural activity is stabilized by inhibition. Mean field models show that UP/DOWN bistability relies on recurrent excitation to maintain an UP state at low levels of external drive. However, it’s unclear how the structure of the excitatory network affects UP/DOWN bistability or transitions between UP and DOWN states. To study UP/DOWN alternations in a balanced network of spiking neurons, we took advantage of an inhibitory plasticity rule (iSTDP) by which inhibitory synapses learn to stabilize activity in postsynaptic cells. Further, we used iSTDP to balance each neuron at a unique target rate. This allowed us to investigate the role of firing rate heterogeneity in DOWN->UP

transitions, which is thought to play a role in NREM function.
Using iSTDP, we built a self-stabilizing network of conductance-based integrate and fire neurons and compared UP/DOWN bistability in networks that were differentiated by the distributions of excitatory synaptic weights and neuronal firing rates. We found that the distribution of excitatory weights strongly influences UP/DOWN bistability in a sparse random network. As is observed in mean field models, strong recurrent excitation was able to maintain a stable UP state in the network. However, with uniformly distributed excitatory weights, UP/DOWN bistability required nonphysiologically strong synapses, which induced large activity fluctuations that destabilized the UP state. When excitatory weights were lognormally distributed, bistability was achieved at reasonable values of synaptic strength. This network was able to show adaptation-mediated UP/DOWN alternations in an excitable regime comparable to that seen during NREM sleep in freely sleeping rats. Further, we found that the behavior of the network at DOWN to UP transitions was strongly influenced by connectivity from the excitatory to the inhibitory population. Dense feedback inhibition prevented excessive synchrony at the DOWN->UP transition and imposed a winner-take-all mechanism in clustered networks. When firing rates were matched to the experimentally observed lognormal distribution, early spiking high firing rate neurons compete to determine the attractor activated at the onset of each UP state. Together, these results reveal key insights to balanced UP/DOWN alternations and are a step toward understanding their role in NREM function.

Disclosures: **J. Gornet:** None. **D. Levenstein:** None. **G. Buzsaki:** None. **J.M. Rinzel:** None.

Poster

081. Network Activity

Location: Hall A

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Program #/Poster #: 081.03/X21

Topic: H.01. Animal Cognition and Behavior

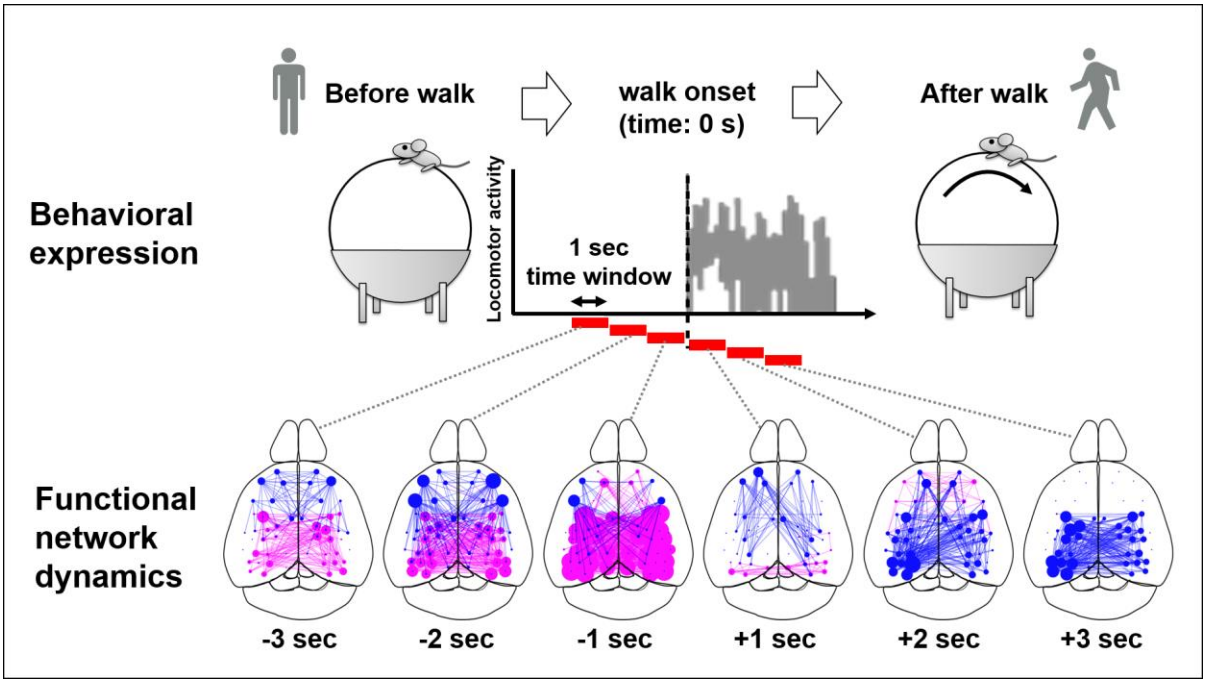
Support: KAKENHI 17H05985
KAKENHI 19H04942
KAKENHI 19K16886

Title: Cortical network dynamics reflect transitions between locomotor states in head-fixed mice in a virtual reality system

Authors: *N. NAKAI, Y. SEKINE, M. SATO, T. TAKUMI;
CBS, RIKEN, Wako, Saitama, Japan

Abstract: The cerebral cortex comprises functional network modules that display dynamic activity patterns that are elicited intrinsically or via external stimuli. The cortical network is considered to contribute to perceptual and cognitive functions and behavioral representations.

However, the way in which the underlying flow of information processed in the cortex drives voluntary behaviors remains unclear at the network level. Currently, the cortical functional network has primarily been studied in sedated resting animals using approaches such as functional MRI. However, this technique is difficult to use in active animals. To overcome this limitation, we monitored cortical network dynamics in mice behaving in a virtual reality (VR) environment using transcranial cortex-wide calcium imaging. In our VR system, a mouse was head-fixed on a spherical treadmill, and interactive VR scenes coupled to the rotation of the treadmill were projected onto a semi-translucent spherical screen. The subject mouse moved in an open field-like virtual arena. The spontaneous locomotion behavior in the VR was simultaneously recorded with cortical activity imaged using calcium imaging. Time-series pairwise correlation between cortical module activities was used to represent the state of the cortical network dynamics. We found that highly organized patterns of cortical network dynamics co-occurred with transitions between two distinct behavioral states (i.e., from resting to walking and vice versa) at a temporal scale of seconds. The network patterns observed could be classified into two behavioral states with approximately 80% accuracy using a support vector machine-based machine learning classifier, indicating that the patterns of the network dynamics provide cortical fingerprints that can predict an animal's locomotor state. Our study demonstrates that patterns appearing in cortical network dynamics in mice have high predictive power for their behavioral states.



Disclosures: **N. Nakai:** None. **M. Sato:** None. **T. Takumi:** None. **Y. Sekine:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Support: KU Leuven C14/17/109
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European Union's Horizon 2020 Framework Programme for Research and Innovation under Grant Agreement No 785907 (Human Brain Project SGA2)

Title: Large-scale cognitive control networks: A monkey fMRI study

Authors: *T. YAO[1], P. BALAN[1], W. VANDUFFEL[1,2,3];
[1]KU Leuven, Leuven, Belgium; [2]MGH Martinos Ctr., Charlestown, MA; [3]Harvard Med. Sch., Boston, MA

Abstract: Despite its importance, the neural mechanisms underlying cognitive control are not yet fully understood. Several models attempt to define it as a set of mutually non-exclusive processes including response selection and suppression, error monitoring, conflict processing and attention.
To study how some of these processes interact throughout large-scale networks, we designed a task in which covert selective attention was cued using two conflicting rules. Monkeys were trained to manually respond to the dimming of a target while ignoring a distractor dimming. At the beginning of each trial, the luminance of the fixation point indicated which rule to use (color or position). Next, the target location was cued either using the spatial location or the color of the same peripheral cue (4 colors for 4 possible locations). These trial types were randomly interleaved together with passive fixation trials in which the subjects simply had to fixate to the central fixation point. The “interaction” between the rules yielded low or high conflict (congruent/incongruent) trials, when the cue color and location indicated the same or different targets, respectively.
As expected, our results showed higher behavioral accuracy and shorter RTs for congruent than incongruent trials. Both cue types activated largely similar cortical networks, although incongruent cues yielded higher fMRI activations in specific parts of LPFC (e.g., FEF, 45, 46) and PPC (e.g., LIP). Noteworthy, wide-spread cortical fMRI suppression was observed when the animals were performing the attention vs. passive fixation trials. Moreover, we found many cortical areas showing stronger suppression during incongruent compared to congruent trials (e.g. premotor cortex and early visual areas V1, V2, V3), suggesting an important role of neural

suppression during conflict processing. More interestingly, we found significant correlations between behavioral accuracy and fMRI responses for incongruent trials that were positive in many visual and prefrontal areas (e.g., V1, V3, V4, MT, MST, V6A, LIP, 46, F7, ACC), but negative in major parts of motor and somatosensory cortex. Strikingly, an almost perfectly anticorrelated pattern was observed during the congruent trials.
In conclusion, the level of conflict between rules shaped both behavior and large-scale fMRI activations and dictated to a surprisingly large extent the interaction between both. Also, suppression of fMRI signals seems to play an essential role in these effects.

Disclosures: T. Yao: None. **P. Balan:** None. **W. Vanduffel:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.05/X23

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 17H06309
KAKENHI 19H01037

Title: Neural dynamics in the mouse secondary and primary motor cortices during self-initiated and externally triggered movements

Authors: *S.-I. TERADA[1], K. KOBAYASHI[2], M. MATSUZAKI[1];
[1]The Univ. of Tokyo, Tokyo, Japan; [2]Natl. Inst. For Physiological Sci., Okazaki, Japan

Abstract: Voluntary movements can be either self-initiated or externally triggered. The higher motor cortex (M2) in primates is activated differently in these scenarios. However, little is known about whether the presence or absence of an external cue primarily determines which type of M2 neural activity is evoked when the same movement is executed in both contexts. To address this, we improved on previously developed mouse forelimb movement tasks (Hira et al., 2014; Terada et al., 2018). We trained head-fixed mice to perform a self-initiated lever-pull task (SI) and an external cue-triggered lever-pull task (ET). Blocks of these tasks (SI and ET blocks) were switched several times within one session. During task performance, we conducted concurrent calcium imaging of layer 2/3 neurons in M2 and M1 using a super-wide-field two-photon microscope (Terada et al., 2018). As expected, the proportion of neurons that exhibited movement-related activity specific to either block was larger in M2 than in M1. Neural population analysis showed that M2 population activity around the onset of lever pull was markedly different between the SI and ET blocks; this could not be explained by slight differences in body movement, including forelimb and orofacial movements, between the blocks. By contrast, the difference in M1 population activity was smaller than that in M2 population

activity and well explained by the behavioral differences. In a majority of trials, M2 population activity showed the post-switch block type immediately after the block switch. However, in some trials, M2 population activity showed the pre-switch block type. In addition, during approximately half of the lever pulls that occurred mistakenly at the interval between the external cues in the ET block, the M2 population exhibited ET block-type activity. These results indicate that the type of M2 population activity evoked depends on both the present and preceding contexts. Both types of M2 population activity presumably evocate the inseparable M1 ensemble whose activity varies to the same extent that the body movement varies.

Disclosures: S. Terada: None. **K. Kobayashi:** None. **M. Matsuzaki:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.06/X24

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant EY029666-01

Title: Multidimensional topography of lateral prefrontal projectome mapped by EM-fMRI in the macaque

Authors: *R. XU, N. P. BICHOT, A. TAKAHASHI, P. K. WEIGAND, A. L. MARINO, R. DESIMONE;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Lateral prefrontal cortex (LPFC) plays an important role in executive control, likely through functionally specific interactions between other parts of the brain and LPFC subregions, e.g., the ventral prearcuate (VPA) region for feature-based visual attention (Bichot et al., 2015). To understand the underlying neural circuitry, it is necessary to first understand what connects to what, i.e., anatomy matters. This remains elusive although many studies have looked at connections of LPFC using anatomical tracers, due to the sparseness of injection sites and individual differences across animals. Here we mapped connections of densely sampled LPFC sites in the same subjects *in vivo*, using combined electrical microstimulation and fMRI (EM-fMRI) in the macaque.
Two macaque monkeys were scanned in 3T magnets under propofol anesthesia. At each site, we collected up to 34 mins of MION signals at 2-mm isotropic resolution. We delivered 500-μA current pulses at 333 Hz for 210 ms per second in 30-sec blocks. The parameters were tuned to optimize contrast-to-noise ratio.
In each animal, we stimulated ~100 sites within up to ~15x20 mm^2 of cortex in caudal-to-mid LPFC, which likely overlaps with areas 8, 45, 46, 9, and 12. Activation patterns can vary

substantially and consistently across stimulation sites only millimeters apart, and were generally consistent with known anterograde, mono-synaptic projections from LPFC. Importantly, we found a surprising degree of topography of LPFC projections. As stimulation sites moved from FEF, for example, toward ventrolateral LPFC, projection sites in the ventral visual stream moved systematically from area V4, posteriorly, through anterior IT areas in the temporal cortex. Moving either dorsally or ventrally from the central portion of this same trajectory of stimulation sites, projection sites shift from IT surface, ventrally, through ventral and then dorsal banks of STS. As in temporal lobe, we found overlapping topographical trajectories in most of the areas LPFC connects to in both animals, including lateral parietal regions in the dorsal visual stream, and anterior cingulate, posterior medial, orbitofrontal, and insular cortices. These trajectories were readily observed from the projection patterns of individual sites, and were confirmed by principal component analysis that summarizes the patterns over many sites objectively. In some sense, the LPFC appears to contain a multidimensional "map" of projections to several other cortical regions. The results suggest that EM-fMRI is a powerful tool for mapping fine-grained patterns of connectivity, which begins to clarify the topographical organization of LPFC connections.

Disclosures: R. Xu: None. **N.P. Bichot:** None. **A. Takahashi:** None. **P.K. Weigand:** None. **A.L. Marino:** None. **R. Desimone:** None.

Poster

081. Network Activity

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Program #/Poster #: 081.07/X25

Topic: H.01. Animal Cognition and Behavior

Support: NSF-NCS 1533623
MIH-NIMH R21 11689422

Title: Context-dependent relationships between locus coeruleus activation, changes in pupil size and coordinated neural activity in anterior cingulate cortex

Authors: *S. JOSHI, J. I. GOLD;
Dept. of Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Ascending neuromodulatory projections from the locus coeruleus (LC) affect cortical neural networks via the release of norepinephrine (NE). However, the exact nature of these modulatory effects on neural activity patterns *in vivo* is not well understood. Here we show that in awake, fixating monkeys, the mean and trial-to-trial variability of firing rates of individual anterior cingulate cortex (ACC) neurons are largely independent of concurrently measured LC activity. In contrast, the correlated variability of pairs of ACC neurons reflects LC activation,

particularly at relatively long timescales (>500 msec). These effects depend on two factors: (1) the mode of LC activation, such that increased tonic (baseline) LC activity coincides with a reduction in ACC pairwise correlations, but external event-driven phasic LC responses coincide with a transient increase in ACC pairwise correlations; and (2) ACC pairwise correlations independent of LC activation, such that tonic LC-linked effects are strongest for positively correlated ACC pairs. Further, we previously showed that changes in pupil diameter covary with changes in LC activity. Here we extend those findings to show that LC-linked changes in pupil diameter also covary with changes in coordinated ACC activity. These results suggest that modulations of signal transmission and information processing that are governed by changes in coordinated activity patterns in cortical networks can, at least in part, result from ongoing, context-dependent changes in arousal that involve activation of the LC-NE system.

Disclosures: S. Joshi: None. **J.I. Gold:** None.

Poster

081. Network Activity

Location: Hall A

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Program #/Poster #: 081.08/X26

Topic: H.01. Animal Cognition and Behavior

Support: JSPS fellowship
NARSAD Young Investigator award
NIMH grant
Simons foundation
Sloan foundation
Klingenstein foundation
Human frontiers science

Title: Prefrontal cortex regulates sensory filtering through a basal ganglia-to-thalamus pathway

Authors: *M. NAKAJIMA, L. I. SCHMITT, M. HALASSA;
MIT, Cambridge, MA

Abstract: To make adaptive decisions, organisms must appropriately filter sensory inputs, augmenting relevant signals and suppressing noise. The prefrontal cortex (PFC) partly implements this process by regulating thalamic activity through modality-specific thalamic reticular nucleus (TRN) subnetworks. However, because the PFC does not directly project to sensory TRN subnetworks, the circuitry underlying this process had been unknown. Here, using anatomical tracing, functional manipulations and optical identification of PFC projection neurons, we find that the PFC regulates sensory thalamic activity through a basal ganglia (BG) pathway. Engagement of this PFC-BG-thalamus pathway enables selection between vision and

audition by primarily suppressing the distracting modality. This pathway also enhances sensory discrimination, and is utilized for goal-directed background noise suppression. Overall, our results identify a new pathway for attentional filtering and reveal multiple roles for this pathway in influencing sensory processing based on internal goals.

Disclosures: **M. Nakajima:** None. **L.I. Schmitt:** None. **M. Halassa:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.09/X27

Topic: H.01. Animal Cognition and Behavior

Support: NIH DP2 Grant

Title: Dynamic motifs capture brain-wide patterns of neural activity

Authors: *C. J. MACDOWELL[1,2], T. J. BUSCHMAN[1];
[1]Princeton Univ., Princeton, NJ; [2]Rutgers RWJ Med. Sch., New Brunswick, NJ

Abstract: Mesoscale imaging of cortical activity has revealed rich dynamics in the spatial-temporal patterns of neural activity. To quantify these dynamics, we leveraged new techniques for unsupervised discovery of repeated spatial-temporal patterns of neural activity across the dorsal surface of the mouse cortex (using widefield calcium imaging). Through this approach, we identified ‘dynamic motifs’ in the spontaneous (resting) neural population activity of awake mice. These dynamic motifs lasted approximately 1 second and engaged several brain regions. Individual brain regions participated in multiple motifs, suggesting the motifs reflected the directed flow of neural activity between different brain regions and not only the correlation of neural activity in multiple regions. The identified motifs were conserved within and between individuals; motifs discovered in one recording session for a given individual explain 87% of the activity of that same individual on a different day and 85% of the activity of a different individual. This suggests the existence of a global, pan-individual repertoire of dynamic motifs. To identify these global motifs, we used unsupervised clustering techniques to cluster the motifs discovered during 5 hours of spontaneous neural activity in 9 adult mice into approximately 19 distinct dynamic motifs with unique spatiotemporal patterns. Together, these global motifs provided a nearly complete basis set of the brain-wide neural activity - they accounted for nearly 90% of the variance of neural activity in 5 hours of held-out data in the same animals. Furthermore, these motifs generalized to capture over 70% of the brain-wide activity in response to sensory stimuli, suggesting that the global motifs don’t simply reflect resting-state activity but also capture more general responses. Further analyses suggested the observed dynamic motifs were influenced by the underlying anatomy: 1) the spatial-temporal patterns of the motifs

corresponded to the underlying anatomical topography of the mouse cortex and 2) spatially similar motifs were less likely to occur in succession than expected by chance. Altogether, our results suggest brain-wide activity is low-dimensional (~19 unique patterns). Of course, these motifs do not capture the specifics of local neural activity (which is likely much higher dimensional). Instead, the brain-wide dynamic motifs may capture the information flow through the brain in support of general cognitive processes (e.g. whisker sensation vs. visual sensation vs. internal processing). The limited number of motifs may reflect an optimal sub-sampling of all possible functional networks of neural populations.

Disclosures: C.J. Macdowell: None. **T.J. Buschman:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.10/X28

Topic: H.01. Animal Cognition and Behavior

Support: T32 NS007433-21
SEARLE SCHOLARS PROGRAM

Title: Diverse activity dynamics of inhibitory subtypes in mouse posterior parietal cortex

Authors: *C. F. KHOURY, C. A. RUNYAN;
Dept. of Neuroscience, Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cortical network dynamics are set and maintained by interactions between multiple excitatory and inhibitory interneuron (IN) subtypes. Vasoactive Intestinal Peptide (VIP), Somatostatin (SsT), and Parvalbumin (PV) are expressed by three non-overlapping populations of INs, which differ reliably in local connection patterns (Pfeffer et al., Nature Neuroscience, 2013). Behavioral state, such as locomotion, modulates activity in these subtypes differently (Fu et al., Cell, 2014). Previously, we discovered that activity correlations ("coupling") within local neural populations are higher in posterior parietal cortex (PPC), an association area, than in sensory cortex. Furthermore, behavioral state modulates the degree of coupling within PPC (Runyan et al., Nature, 2017). Here we investigate contributions of diverse IN subtypes to PPC activity correlations by comparing coupling of VIP, SsT, and PV neurons during spontaneous running behavior. We hypothesize that IN subtypes differ in their coupling to neurons of the same or different subtypes. We used two-photon calcium imaging to measure spike-related activity in populations of neurons in PPC of male and female mice running voluntarily on a spherical treadmill. We identified PV, SsT, or VIP neurons using Cre-dependent expression of tDTomato or Channelrhodopsin. We estimated the extent to which each neurons' activity was

coupled to the activity of neighboring neurons by comparing the prediction performance of two versions of an encoding model that we developed (Runyan et al., Nature, 2017). The uncoupled version of the model predicted each neuron's activity using only behavior-related variables, e.g. running speed, while the coupled version of the model included behavior-related predictors as well as the activity of simultaneously imaged neurons. The coupling predictors consisted of neurons within the same cell type (e.g. PV neurons for a PV neuron), neurons outside of cell type, or all simultaneously imaged neurons. Preliminary results suggest that PV, SsT, and VIP neurons differ markedly in coupling within and across subtypes. These data illustrate the diverse, cell-type specific activity dynamics, which govern PPC network activity.

Disclosures: C.F. Khoury: None. **C.A. Runyan:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: R01-MH55806
P30-EY08126
U54 HD083211
Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Microcircuitry of agranular cortex: Multiplexed executive control and performance monitoring signals

Authors: *S. P. ERRINGTON, A. SAJAD, J. D. SCHALL;
Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Medial frontal cortex contributes to response monitoring and executive control, but the microcircuitry enabling this function is unknown. We recorded neural spiking across all layers of supplementary eye field, an agranular medial frontal area, in monkeys performing a saccade-countermanding (stop signal) task. Neurons with different profiles of activation had different spike widths, time courses of activation, and were concentrated differently across layers. Neurons responding to visual cues were a mixture of shorter-latency narrow spikes and longer-latency wide spikes concentrated in layers 2 and 3 (L2/3) and in upper layer 5 (L5). Other neurons were modulated differentially only after response inhibition was accomplished. Neurons with transient responses proportional to response conflict were predominantly wide spikes found in all layers. Neurons enabling responses most often had wide spikes and were concentrated in L2/3 and L5, while neurons disabling responses were most often narrow spikes and were more concentrated in L2/3. Relative to those in L5/6, neurons in L2/3 were more likely to modulate

during multiple task phases, carrying multiple signals. Relative to those with wide spikes, neurons with narrow spikes were more likely to carry multiple signals. These findings complement previous description of the laminar organization of neurons signaling error, reward gain, and reward loss, reveal novel features of cortical microcircuitry supporting response monitoring, extend understanding of agranular cortical organization, and expose limits of the mixed-selectivity perspective.

Disclosures: S.P. Errington: None. **A. Sajad:** None. **J.D. Schall:** None.

Poster

081. Network Activity

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Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.12/X30

Topic: H.01. Animal Cognition and Behavior

Support: R01-EY019882

Title: Microcircuitry of agranular frontal cortex: Laminarphase-amplitude coupling for cognitive control

Authors: *R. DOUBNIA[1], A. SAJAD[2], B. HERRERA[1], J. SCHALL[2], J. RIERA[1], G. WOODMAN[2];
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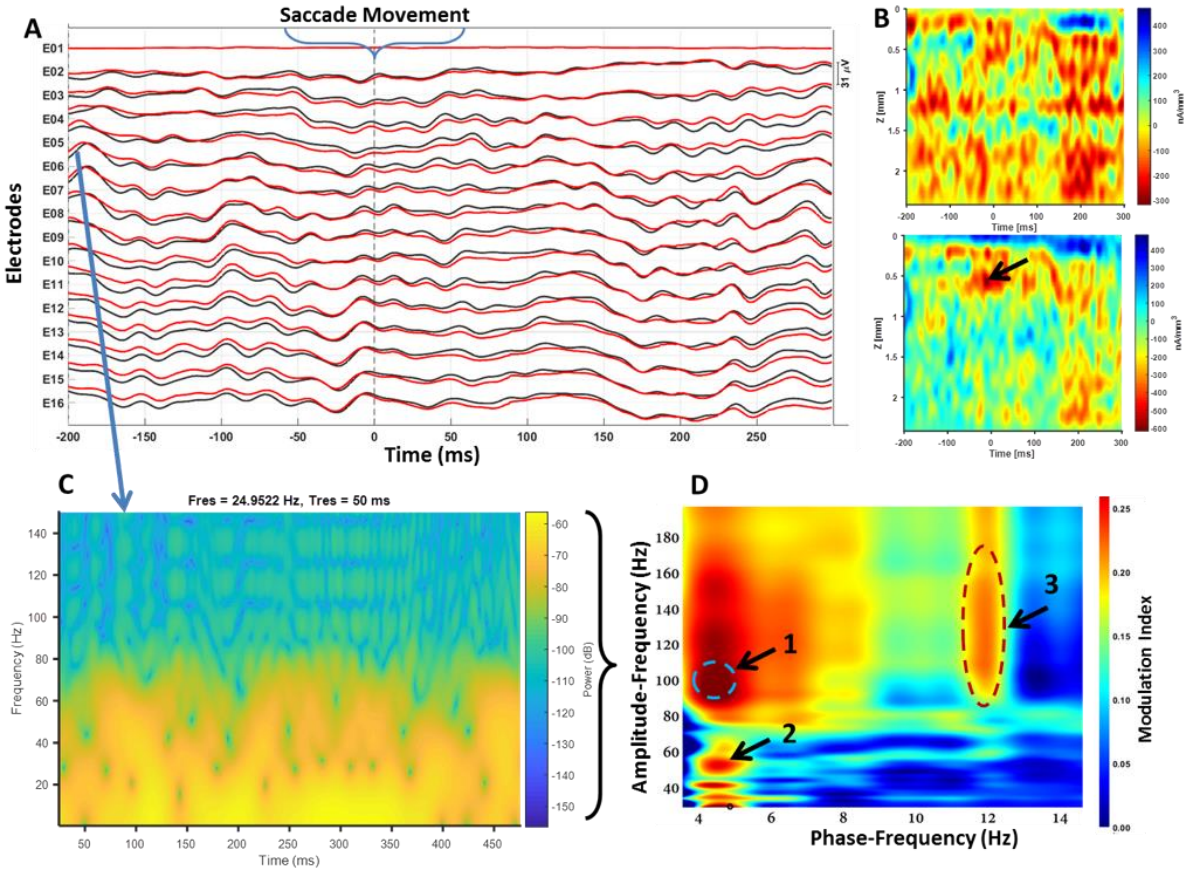
Abstract: An understanding of the circuitry accomplishing executive control depends on knowledge about the dynamics of local field potentials (LFP) across cortical layers. Changes in the LFP cross-spectral/phase composition have been demonstrated to be relevant in cross-cortical communication in the agranular supplementary eye field (SEF, Ninomiya et al. 2015 J Neurophysiol), a critical cortical area in performance monitoring of saccades. Specifically, Ninomiya et al. demonstrated the existence of a laminar organization in both the spectral content (SC) and in the phase-amplitude coupling (PAC) effect in SEF locked to ongoing alpha oscillations. The spatial profiles of the SC and PAC differ from those observed in primary visual cortex (V1). The frequencies showing a PAC effect were also different from those reported by Spaak et al. (2012 Curr Biology) for V1. However, the SC and PAC analyses in SEF by Ninomiya et al. were performed during resting state. Here, we report data obtained in macaque monkeys performing a saccade countermanding task that has been used in numerous previous investigations of cognitive control. Our goal is to assess whether or not similar effects are present in this task; however, the randomness of the saccade response time relative to target presentation lead to new challenges for these analyses. Particularly, correction of this randomness is crucial for the Fourier analysis as the mean statistic (i.e., the event-related LFP) should be previously removed from each trial. An already developed method by our lab (Riera et al., 1995, Int J

Biomed Comp) is applied to estimate the saccade event related response after having corrected for reaction-time variability. An accurate estimation of event-related LFP (Fig. 1A) is also necessary for the proper interpretation of results from the current source density (CSD) analysis. CSD maps generated with and without such a variability correction are compared (Fig. 1B). Finally, layer-dependent SC (Fig. 1C) and PAC (Fig. 1 D) analyses in SEF are performed after reaction-time variability had been corrected.

Funding: R01-EY019882

Figure 1



Disclosures: **R. Doubnia:** None. **A. Sajad:** None. **B. Herrera:** None. **J. Schall:** None. **J. Riera:** None. **G. Woodman:** None.

Poster

081. Network Activity

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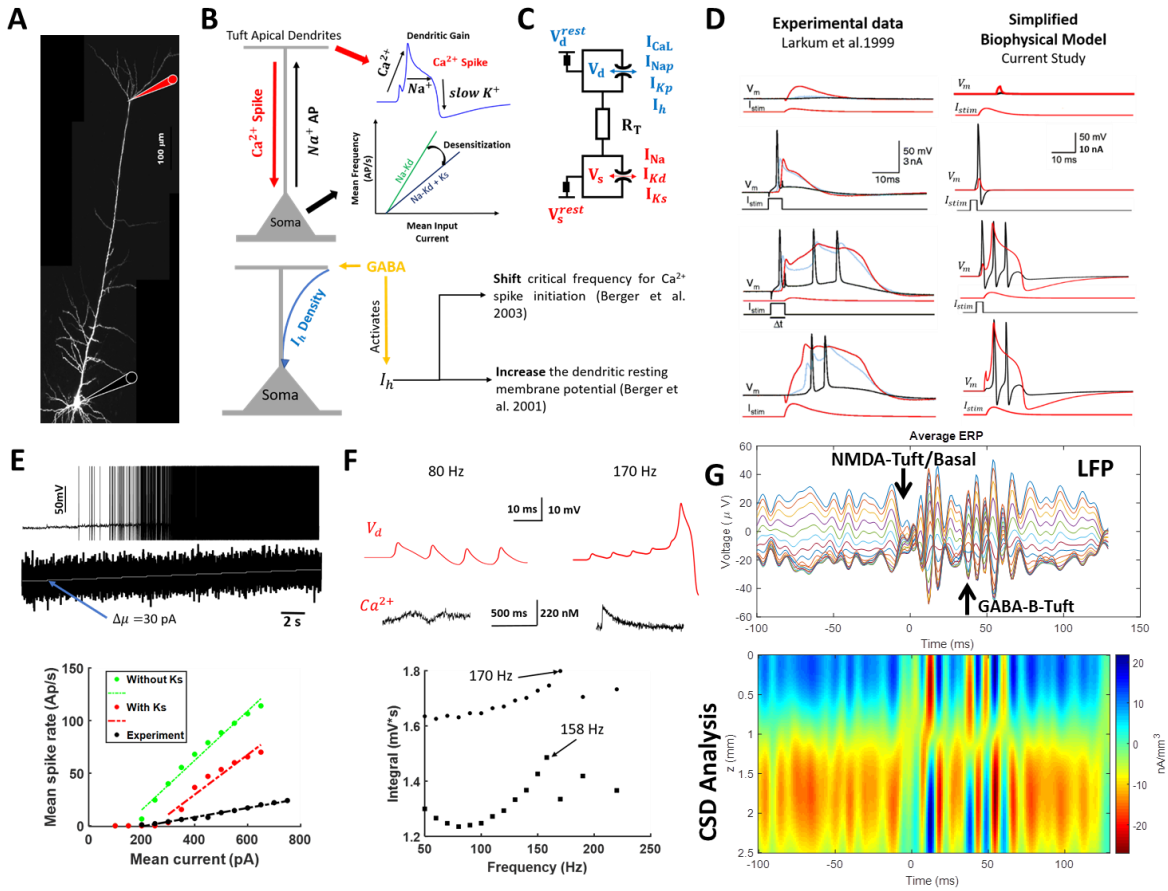
Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01-EY019882

Title: Microcircuitry of agranular frontal cortex: A stochastic 2-compartment model of neocortical pyramidal cells

Authors: *B. HERRERA[1], A. SAJAD[2], G. F. WOODMAN[3], J. D. SCHALL[4], J. J. RIERA[1]; [1]Biomed. Engin., Florida Intl. Univ., Miami, FL; [2]Psychological Sci., [3]Dept Psychol, [4]Psychology, Vanderbilt Univ., Nashville, TN

Abstract: An understanding of the circuitry accomplishing executive control depends on knowledge about the properties of neocortical pyramidal cells (PCs) (Fig. 1A). Highly-complicated (hundreds of compartments and dozens of ionic channels) models have been proposed to account for the Ca^{2+} spike-dependent gain and the existence of the critical frequency for Ca^{2+} spike genesis in PCs (Hay et al., 2011; Bahl et al., 2012; Mäki-Marttunen et al., 2018). However, other features of PCs have not been explained yet with a theoretical model. Moreover, it has not been possible to evaluate their impact on large population dynamics due to their high computational cost. In this study, we present a simple stochastic 2-compartment model of PCs that reproduces all three principal features (B-C). The model comprises a combination of Na^+-K^+ conductance and AHP (Ca^{2+}-dependent K^+ current) for the soma/basal-dendrites. The dendritic tuft includes the persistent Na^+, the hyperpolarization-activated cation (Ih), the slow inactivation K^+ channel and the Ca^{2+} L-type channel. As for the calcium active control in the dendritic tuft, we assumed single Michaelis-Menten kinetics. Our model was able to replicate the three major phenomena in this type of cells: **a)** the back-propagating action potential (AP) activated Ca^{2+} spikes underlying the above-referred changes in dendritic gain (D), **b)** the AHP-dependent desensitization of their soma (E), and **c)** the shifting of the critical frequency via activation of the Ih current (F), which could be enhanced by GABA-ergic synaptic activity. The results demonstrate that this simple neuronal model can serve as a powerful tool to study the cortical organization of agranular areas, such as the supplementary eye field (SEF). To illustrate the usefulness of our model in this area, we simulated local field potential (LFPs), and calculated the related current source density (CSD) maps, evoked by the activation of an agranular cortical column via NMDA inputs at the apical/basal dendrite and a delayed apical inhibition from intra-cortical connections (G).



Disclosures: **B. Herrera:** None. **A. Sajad:** None. **G.F. Woodman:** None. **J.D. Schall:** None. **J.J. Riera:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: R01-MH55806
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P30-EY08126
Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Microcircuitry of agranular cortex: Laminar organization of signals for the feedback related negativity

Authors: *A. SAJAD, J. D. SCHALL;
Vanderbilt Univ., Nashville, TN

Abstract: Cognitive control involves monitoring the cost of errors and value of rewards in relation to expectations (prediction error). These are indexed electrophysiologically by the feedback related negativity (FRN), which originates in medial frontal cortex (mFC). However, the underlying microcircuitry is unknown. We sampled neurons across all layers of an agranular area in mFC, known as supplementary eye field. Neural discharges were recorded from two monkeys while performing the stop-signal task. On most trials, monkeys were rewarded for looking at a peripheral visual stimulus to the right or to the left, but occasionally a stop-signal instructed them to inhibit the movement. Stop-signal timing was adjusted to ensure failure to stop on ~50% of stop-signal trials. Importantly, each direction was associated with low or high reward amounts and this association flipped unpredictably after ~20 rewarded trials in each block. As expected, both monkeys showed sensitivity to differential reward amounts. The response time (RT) for saccades to high-reward targets was significantly faster than that for low-reward targets. Upon reward association reversal, RT adaptation was observed with progressive reduction of RT to the high-reward target, and increase to the low-reward target. Previously, in a sample of 575 units, we characterized the laminar organization of neurons that signaled error (Error neurons), reward gain (Gain neurons), and lack thereof (Loss neurons) (Sajad et al., Nat Neurosci, 2019). Here, we show that Error, Gain, and Loss neurons show sensitivity to the expected reward value. Error neurons in layers 2 and 3 (L2/3) show greater sensitivity to the cost of error compared to those in L5/6. Gain neurons, which are more common in L5/6, show increased facilitation for larger reward amounts, whereas Loss neurons, particularly those in L2/3 exhibit increased suppression for larger reward amounts. Comparison of neural discharge rate between early and late RT-adaptation trials revealed neurons that signaled reward prediction error (RPE). Neurons signaling positive and negative RPE were distributed across all layers. However, neurons exhibiting response facilitation for negative RPE were exclusively pyramidal neurons largely confined to L2/3, while those exhibiting suppression were an intermix of interneurons and pyramidal neurons with a relatively larger proportion in L5/6. These results complement previous reports, reveal the laminar microcircuitry of cognitive control signals for value and surprise encoding in agranular mFC, and establish a cortical substrate for FRN.

Disclosures: A. Sajad: None. **J.D. Schall:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC CREATE IRTG 449313-2014
CIHR FDN-148418

Title: Laminar organization of reward signal in the superior colliculus priority map

Authors: *J. Y. KAN[1], B. J. WHITE[1], L. ITTI[2], D. P. MUNOZ[1];
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Computer Sci. Dept., USC, Los Angeles, CA

Abstract: Visual orienting is sometimes modeled as being directed by a saliency map representing the conspicuity of visual space, and a priority map that combines bottom-up saliency and top-down relevance. We hypothesize that the superior colliculus (SC) embodies the role of a saliency map and a priority map compartmentalized in the superficial (SCs) and intermediate (SCi) layers, respectively. Using a multi-channel linear micro-electrode, we compared monkey SCs and SCi firing rate and local field potential (LFP) in response to visual saccade targets that were matched for saliency but varies in relevancy. One or two bar-shaped stimuli serving as saccade targets were presented as salient pop-out stimuli in a homogenous wide-field array of bars that differ from targets in color and orientation. Target color was used to indicate large, small, or no reward if the monkey makes a saccade to it, varying the behavioural relevance and therefore priority of the targets. We found a depth-dependent representation of priority in the SC. There were only small differences in SCs firing in response to the various stimuli. In contrast, SCi activity showed strong modulation starting around 80ms after stimuli appearance, with rewarded stimuli evoking the highest response , followed by non-rewarded but salient stimuli, and homogenous background stimuli evoking the lowest response. This pattern of reward modulation was qualitatively similar even when controlling for saccade direction. A stimulus-evoked biphasic LFP response was observed in both layers of the SC, with reward modulation emerging in the later, negative portion of the response. Our observations are consistent with our hypothesis that top-down relevance in the form of reward signal has a greater representation in the SCi than in the SCs.

Disclosures: J.Y. Kan: None. **B.J. White:** None. **L. Itti:** None. **D.P. Munoz:** None.

Poster

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Support: Australian Research Council grant DP140101968
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International Neuroinformatics Coordinating Facility, Seed Grant

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Title: The marmoset mesoscale connectome

Authors: *P. THEODONI[1,2,3], P. MAJKA[4,5], D. H. RESER[6], M. G. ROSA[7,5], X.-J. WANG[1]; [1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]NYU-ECNU Inst. of Brain and Cognitive Sci., Shanghai, China; [3]New York Univ. Shanghai, Shanghai, China; [4]Lab. of Neuroinformatics, Nencki Inst. of Exptl. Biol., Warsaw, Poland; [5]Ctr. of Excellence for Integrative Brain Function, Australian Res. Council, Canberra, Australia; [6]Monash Rural Hlth., Monash Univ., Churchill, Australia; [7]Biomedicine Discovery Inst. and Dept. of Physiol., Monash Univ., Melbourne, Australia

Abstract: Cognitive processes such as decision-making involve multiple interacting brain regions, the dynamical operation of such a large-scale brain system remains poorly understood. To make progress, quantitative characterization of the structural connectivity of parcellated areas is essential. Here we analyze a consistently collected dataset of weighted and directed cortico-cortical connectivity of the marmoset monkey (Callithrix jacchus), along with the laminar distribution of the projections and the interareal wiring distances. The dataset, available through the Marmoset Brain Connectivity Atlas portal (http://marmosetbrain.org), comprises the results of 143 retrograde tracer injections revealing all the cortical inputs, and their laminar origin, to 55 out of the 116 total areas broadly distributed across the marmoset cerebral cortex. Our analysis reveals many features of the cortical architecture that have been preserved throughout millions of years, such as the highly heterogeneous log-normally distributed connectivity weights, the broadly distributed in- and out- degree, the three motif and clique distribution, the normally distributed wiring distances, and the decay of the probability of connections as a function of the similarity distance. In addition, there are cortico-cortical connectivity properties that scale with the brain size, such as the smaller the brain the longer the projections and fewer the week connections, or they are species-dependent such as the density of the connectivity matrix. The laminar distribution of the inputs defines the hierarchy in which motor/premotor areas are found on the top of the marmoset cortical hierarchy, higher than prefrontal areas, challenging our way of viewing the integration of inputs and information flow. In addition, sensory streams are segregated, and association areas are grouped with them. Finally, microstructural features, such as spine count and neural density are highly inversely correlated and correlate with the hierarchy, providing a direct link of different scales within the cortex essential for the understanding of the brain function as a whole via theoretical modeling.

Disclosures: **P. Theodoni:** None. **P. Majka:** None. **D.H. Reser:** None. **M.G. Rosa:** None. **X. Wang:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: the Simons Collaboration on the Global Brain program grant 543057SPI
NIMH MH062349
ONR Grant N00014-17-1-2041

Title: Understanding distributed working memory using a large scale circuit model of the mouse cortex

Authors: *X. DING[1], S. FROUDIST-WALSH[1], D. BLISS[2], X.-J. WANG[1];
[1]New York Univ., New York, NY; [2]Ctr. for Neural Sci., NYU, New York, NY

Abstract: Working memory is the brain's ability to actively maintain and manipulate information internally in the absence of sensory stimulation. The prefrontal cortex has been identified as a candidate neural substrate for working memory. However, emerging evidence suggests working memory is not stored only in a single area, but we currently know little about the mechanisms and principles of distributed working memory in a multi-regional brain circuit. Here we propose a large-scale computational model for mouse cortex which is constrained with different sets of experimental data at each level of description. Each cortical area is modeled as a network of interacting excitatory and inhibitory neurons. The inhibitory strength changes across areas according to the density of parvalbumin-expressing neurons measured by anatomical studies. In addition, a newly proposed MRI-based macroscopic hierarchical gradient is introduced to specify the feedforward and feedback relationship between areas. The large-scale circuit model obtained with this method consistently reproduces persistent activity in frontal areas and predicts that the persistent activity also appears in insular cortex and cingulate cortex. Furthermore, our model predicts that the intrinsic time scale in each brain area correlates with the macroscopic gradient in the mouse cortex. Our model provides a computational platform to investigate the large-scale nature of cognitive functions.

Disclosures: **X. Ding:** None. **S. Froudist-Walsh:** None. **D. Bliss:** None. **X. Wang:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH062349

Title: A gradient of cortical dopamine stabilizes distributed working memory representations in a large-scale model of macaque cortex

Authors: *S. FROUDIST-WALSH[1], N. PALOMERO-GALLAGHER[2], D. P. BLISS[1], X. DING[1], K. KNOBLAUCH[3], L. JANKOVIC-RAPAN[2], M. NIU[2], H. KENNEDY[3], K. ZILLES[2], X.-J. WANG[1];
[1]New York Univ., New York, NY; [2]Res. Ctr. Juelich, INM-1, Juelich, Germany; [3]Stem Cell & Brain Res. Inst., Inserm U846, Bron, France

Abstract: Working memory is a core component of cognition in many everyday situations, from memorizing a phone number to remembering your friends' orders at the ice-cream truck. In most real-life situations, this memory must be maintained in spite of a variety of other distracting stimuli. How do we prevent these distractors from affecting our behavior? Distractor-resistance relies on the executive aspects of working memory, including focused attention and inhibitory control, and can be improved by pro-dopaminergic drugs. Dopamine increases the ratio of inhibition targeting the dendrite relative to the cell body of pyramidal neurons, but it is not known how this local synaptic action of dopamine affects distributed cortical representations of stimuli during working memory. Here we describe a hierarchical gradient of dopamine D1 receptor expression per neuron across monkey cortex, measured using *in-vitro* autoradiography, ranging from low expression in early visual cortex to high expression in associative areas of frontal and parietal cortex. We have developed a large-scale dynamical model of monkey cortex, with dopamine modulating interactions between cell types to a greater degree in those areas with a high dopamine receptor density. Areas are connected in the model using weighted-and-directed connectivity data from retrograde tract-tracing experiments. We find that distracting stimuli are always temporarily represented in sensory and parietal areas. However, we show that the level of dopamine release can flexibly control whether distracting stimuli are propagated to frontal cortex to be maintained in working memory in a distributed cortical network. Virtual lesions to dorsolateral prefrontal cortex (dlPFC) resulted in less robust working memory representations. We simulated the systemic application of a D1-receptor agonist as a potential treatment for the working memory loss associated with dlPFC injury. Distractor-resistant working memory activity was restored for lower levels of the drug, but high levels of the drug led to unstable working memory representations. This work provides a way forward to link findings from

systems neuroscience and neuroanatomy of large-scale brain circuits to treatment for patients with working memory dysfunction.

Disclosures: **S. Froudist-Walsh:** None. **N. Palomero-Gallagher:** None. **D.P. Bliss:** None. **X. Ding:** None. **K. Knoblauch:** None. **L. Jankovic-Rapan:** None. **M. Niu:** None. **H. Kennedy:** None. **K. Zilles:** None. **X. Wang:** None.

Poster

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Program #/Poster #: 081.19/X37

Topic: H.01. Animal Cognition and Behavior

Support: BranchOut Neurological Foundation
NSERC
CIHR

Title: Reorganization of neural encoding in the retrosplenial cortex by the potent psychedelic ibogaine

Authors: ***V. E. IVAN**[1], I. M. ESTEVES[1], A. R. NEUMANN[1], M. MOHAJERANI[1], B. L. MCNAUGHTON[1,2], A. J. GRUBER[1];
[1]Canadian Ctr. for Behavioral Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada;
[2]Neurobio. and Behavior, Univ. of California at Irvine, Irvine, CA

Abstract: Ibogaine is a psychoactive indole alkaloid that has been used for many decades as a treatment for severe opioid dependency in alternative rehabilitation centers. Preclinical studies in a variety of animals have shown that acute ibogaine administration reduces self-administration of abused drugs well after the drug has washed out, suggesting some enduring plastic change in the brain. Virtually nothing, however, is known about how this drug affects encoding of information in the brain related to reward seeking, such as location and reward processing. Here, we investigate acute and lasting effects of ibogaine on information encoding in the retrosplenial cortex (RSC), a region of neocortex involved in action planning and navigation. Some RSC neurons have activity patterns that strongly resemble hippocampal CA1 place cells, showing robust firing fields that correlate with location (Mao, 2017), which can be used to asses information encoding. We used 2-photon $^{Ca2+}$ imaging to record the acute and lasting effects of ibogaine on the RSC in head-fixed mice trained on a treadmill task. Mice received a drop of sucrose solution on every lap of the treadmill belt. For each daily session, animals were recorded on task for 10 minutes, and then given a subcutaneous injection. Mice received two days of saline injections (for habituation), and then either 3 days of ibogaine HCl (40 mg/kg; n=6) or 3 days of saline (n=2). Our results show that ibogaine HCl significantly decreased the number of

"place cells" found in the superficial layer of the RSC, decreased the specificity of the remaining place cells, and increased the cell firing rates. Recordings 24 hours after the final drug administration showed that rates of activity were consistent with pre-drug conditions, but that the number and specificity of place cells remained altered. These data suggest that ibogaine has a pronounced acute effect on cortical neural dynamics, and a lasting effect on the brain that appears to partially reset the spatial representation of the task in RSC.

Disclosures: V.E. Ivan: None. **I.M. Esteves:** None. **A.R. Neumann:** None. **M. Mohajerani:** None. **B.L. McNaughton:** None. **A.J. Gruber:** None.

Poster

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Program #/Poster #: 081.20/X38

Topic: H.01. Animal Cognition and Behavior

Support: NIMH R37MH087027
The MIT Picower Institute Innovation Fund

Title: Achieving and using stability in neural circuits

Authors: *L. KOZACHKOV[1], M. LUNDQVIST[1], J.-J. SLOTINE[2], E. K. MILLER[1];
[1]Brain and Cognitive Sci., [2]Mechanical Engineering, Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: The brain must deal with neural networks that are highly dynamic and noisy. Neural activity fluctuates highly from moment to moment and varies considerably between experimentally identical trials. These fluctuations in network state can be due to a variety of factors including variability in membrane potentials, inputs, synaptic changes due to recent experience, etc. Yet, in spite of these fluctuations networks must eventually manage to achieve stability. Despite being "knocked around" by different perturbations and noise, networks must eventually reach a highly consistent state—or sequence of states (a trajectory)—for their computations to make sense. To understand this, we studied a dynamic form of stability called contractive stability, developed in the control theory literature. Unlike a chaotic system where perturbations and distortions can be amplified over time, the trajectories of a contracting system through state space will converge towards the same path thus achieving stability.
We therefore applied contraction analysis to biologically realistic networks (recurrent neural networks, RNNs, with plastic synapses and receiving time-varying inputs). This revealed several classes of synaptic plasticity and connectivity that naturally produced contraction, including anti-Hebbian plasticity and sparse connectivity. We then used these forms of plasticity in RNNs to examine how contracting networks can perform functionally relevant computations in the

presence of noise and disturbance. These computations included context-dependent sensory integration and retaining stimuli in working memory. These results pave the way for future brain modeling studies which seek to understand how information is processed not just within individual neural circuits but distributed across multiple plastic and input-driven circuits.

Disclosures: L. Kozachkov: None. **M. Lundqvist:** None. **J. Slotine:** None. **E.K. Miller:** None.

Poster

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Title: Frontal cortex dynamics during passive sequence viewing using behaving nonhuman primate fMRI

Authors: *N. YUSIF RODRIGUEZ, D. BASU, T. M. DESROCHERS;
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Abstract: Keeping track of and detecting changes of sequential visual stimuli is necessary for daily life. For example, when riding the bus, you must track the series of buildings you pass to identify and get off at the correct stop. These non-motor or abstract visual sequences demand that we internally monitor sequential order. This monitoring is supported by rostrolateral prefrontal cortex (RLPFC) in humans. RLPFC activity increases or ramps up through abstract sequential tasks, and these dynamics are necessary for sequential task performance (Desrochers et al. 2015, 2019). Nonhuman primates monitor sequential stimuli in their environment, but it is unknown if ramping dynamics in a similar region supports this function. Passive monitoring of sequential auditory stimuli engages a network of brain regions that overlap across human and nonhuman primates (Wang et al. 2015). To determine if the passive monitoring of visual stimuli 1) engages a similar network of areas as auditory stimuli, and 2) engages ramping dynamics in the PFC in nonhuman primates as were observed in humans, we designed a visual passive sequence monitoring task. Nonhuman primates were habituated to four-item visual sequences while fixating in the fMRI. After habituation, they were exposed to rare deviants from the habituated

sequences. Initial data showed activations in response to visual sequence deviants similar to those observed in the auditory task (Wang et al. 2015). Further, ramping activation was observed in the PFC during the passively monitored sequences. However, multiple processes could underlie the observed ramping. The sequences were composed of image and timing patterns. To test the factors that elicit this ramping activation and the brain areas involved, we created a set of tasks in which each these characteristics were isolated. We hypothesize that image patterns will elicit ramping in the monkey PFC, similar to our initial findings and previous human studies. Further, we hypothesize that ramping may occur in conditions where there is a timing pattern, but not in the same network of areas as when the images are patterned. When there is no image or timing pattern, we predict that will be no ramping activation. Preliminary results from these new tasks suggest that there is ramping in the PFC in response to patterns of images, with or without timing. These results suggest that the patterns of images are the salient sequential features that underlie the tracking of sequence information in the PFC of nonhuman primates. Future work will determine if this result is unique to image pattern and how it relates to similar dynamics in the human frontal cortex.

Disclosures: **N. Yusif Rodriguez:** None. **D. Basu:** None. **T.M. Desrochers:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.22/X40

Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI 16H02061
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JSPS KAKENHI 16K18380

Title: Dynamic encoding of choice-target information in the perirhinal cortex

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Abstract: Cortical neurons flexibly respond to multiple task-events characterized by distinct computational aspects, such as cue, action and reward. There are two possible explanatory scales for this property, dynamic population code achieving computational flexibility and coherent single-neuron code associating relevant information throughout different task-events.
To synthesize our understanding of these independently developed hypotheses, we analyzed neural responses in the perirhinal cortex (PRC) in two epochs, where different computations were demanded: making prediction about choice outcomes (cue epoch) and reinforcing the choices (reward epoch). We trained rats (n = 5) in a standard two-alternative forced-choice task,

where the animals chose a target port (left/right) associated with a presented cue (visual/olfactory) to obtain water reward. We recorded spiking activities of over two-hundred neurons in the PRC. To understand population structure during the cue and reward epochs, we performed principal component analysis for the population responses. We identified two-dimensional neural subspaces independently for those epochs, where the cue-modality and choice-target (that is, a target port which the animals chose in each trial) information was represented. We found that first dimension of the cue-epoch subspace was moderately correlated with first dimension of the reward-epoch subspace, serving as a shared neural dimension where choice targets were consistently discriminated through the different epochs. Importantly, this dimension was organized by dynamic contribution of individual neurons between the epochs rather than static choice-target selectivity at single-neuron level, indicating interaction between coherent single-neuron responses and population dynamics. Interestingly, in the shared dimension, the discriminability of the choice targets was correlated with discriminability of the different task-epochs. We also revealed that the integrated discriminability of choice targets and task epochs critically depended on coherent temporal response patterns at individual neurons. These findings indicated the reconciled temporal dynamics and coherence at different levels, suggesting its potential role in typical functions of the PRC including recognition and episodic memory.

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Poster

081. Network Activity

Location: Hall A

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Program #/Poster #: 081.23/X41

Topic: H.01. Animal Cognition and Behavior

Title: Cortical encoding of attentional set-shifting abilities

Authors: *F. SCARSI[1], S. GUADAGNA[1], D. DAUTAN[1], R. MASTROGIACOMO[1], D. SCHEGGIA[1], M. NIGRO[1], T. BALLARD[2], U. OLCESE[3], F. PAPALEO[1];
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Abstract: The Higher Order Cognition (**HOC**) is a complex and multi-facets function that require controlled, strategic or executive processes. The HOC refers to the mental processes of reasoning, decision making, problem-solving, planning, etc. *(*Collins & Koechlin, 2012; Lunt, et al., 2012; Necka & Orzechowski, 2005*)*. The prefrontal cortex (PFC) is one of the major hubs involved in the mediation of higher-order cognitive abilities. This brain area receives consistent dopaminergic inputs (*Puig et al., 2014*), indeed, dopamine signaling in the PFC has a key role in

the modulation of higher-order cognitive functions. In particular, dopamine/D2 signaling within the PFC might be implicated in the causes and treatment of cognitive deficits evident in schizophrenia. Here, we investigated the cognitive and related PFC electrophysiological effects of clinically-relevant genetic variants altering D2 receptors.We used three mutant mice with the alteration of the D2 signaling: Dysbindin +/- mice, with an overexpression of cortical D2 receptors, D2L +/- mice, with an unbalance of D2 isoforms, and the combination of these genetic variants. These mice were tested in a recently validated automated Intra-/Extra-Dimensional Attentional Set-Shifting task for mice (Scheggia et al Biol Psy 2014, Scheggia et al Nat Comm 2018), while performing in vivo oximetry or extracellular electrophysiological recordgins in freely moving conditions. We found that, while wild type (WT) animals show an increase in both oxygen consumption and neuronal activity, particularly during the extra-dimensional shift (EDS) following correct response, the same effect was altered in mutant models. The concurrent alteration of both genes differentially impacted mice behavior together with their respective cortical activity across the different stages. These findings support and expand the role of Dys and D2L\D2S receptors in executive functions and provide evidence for specific associations between changes in D2 signaling and electrical activity in the mPFC and cognitive performance.

Disclosures: **F. Scarsi:** None. **S. Guadagna:** None. **D. Dautan:** None. **R. Mastrogiacomo:** None. **D. Scheggia:** None. **M. Nigro:** None. **T. Ballard:** None. **U. Olcese:** None. **F. Papaleo:** None.

Poster

081. Network Activity

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 081.24/X42

Topic: H.01. Animal Cognition and Behavior

Title: Longitudinal functional and behavioral markers of trait versus state individual characteristics

Authors: *A. EICHENBAUM[1], I. PAPPAS[1], D. J. LURIE[2], J. R. COHEN[3], M. D'ESPOSITO[1]; [1]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA; [2]Psychology, Univ. of California, Berkeley, Berkeley, CA; [3]Dept. of Psychology and Neurosci., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Functional connectivity neuroimaging data collected at rest are sensitive to a diverse set of psychobiological factors, including age (Chan et al., 2014), disease (Finn & Constable, 2016), and cognitive ability (Chen et al., 2016). However, questions remain about the extent to which features of resting state functional connectivity are trait markers that remain largely stable across time and contexts (Gratton et al., 2018), or are indicative of an individual's state that varies over time (Cohen, 2018). Moreover, the distinction between trait vs. state may best be

captured by utilizing measures of static vs. time-varying functional connectivity (TVC). Thus, in this study, resting state fMRI (rs-fMRI) data was analyzed using both types of measures to further delineate the influence of state and trait on functional connectivity. Twenty-three healthy subjects (10 female, mean age: 28.3yrs) completed up to three sessions during which rs-fMRI, cognitive task (outside the scanner), and self-report questionnaire data were collected. Sessions 2 and 3 occurred one week and one year after session 1, respectively. Each session consisted of two 6-minute rs-fMRI scans during which subjects fixated on a crosshair. In order to assess the variability of functional connectivity across time, static functional connectivity matrices were clustered across subjects and sessions using an agglomerative hierarchical clustering algorithm. Behavioral data were clustered and analyzed in a similar manner. Next, measures of TVC were computed by fitting a hidden Markov model to the concatenated rs-fMRI time-series across all subjects and scans. Finally, we used a canonical correlation analysis to find pairs of canonical variates ("modes") that maximized the correlation between functional connectivity (static vs. time-varying) and behavior. Overall, static functional connectivity matrices and behavior were more similar within subjects than between subjects at similar sessions. Using a canonical correlation analysis, we found a single significant mode between static functional connectivity and behavior. Replicating previous TVC studies, the hidden Markov model analysis revealed a hierarchical temporal organization of subjects' TVC into two clusters of states. Unlike static connectivity, the canonical correlation analysis of TVC and behavior revealed two significant modes of covariation. The two modes found with TVC relate differentially (positive vs. negative) to the mode found with static connectivity, indicating that TVC may uncover relationships with behavior not found with static functional connectivity measures.

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Poster

081. Network Activity

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Title: Time reversal in the relationship between neural activity and behavior provides a signature of executive control in the mouse medial prefrontal cortex

Authors: J. AFONSO[1], I. PICA[1], A. MONTEIRO[1], C. GOLDEN[2], P. CHADDERTON[2], S. ROYER[3], ***A. RENART**[1];
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Abstract: Executive function, defined as the selection and monitoring of behaviors that facilitate the attainment of goals in a particular environment, is orchestrated by the prefrontal cortex. In many instances, the estimation of the state of the environment requires integration of movement signals generated by the body - functioning as input to the executive controller - whereas goal attainment through motor output requires the control of these same movement signals - functioning here as the output of the controller. We studied this process in a delayed response task where head-fixed mice in darkness had stop or not at a target location (TL) in the belt (marked by a change in texture) contingent on the identity of a auditory cue presented at a various distal locations in the treadmill. Mice slow down before the TL in preparation for the stopping response after the appropriate cue, providing evidence that they estimate their position in the belt. Recordings of the simultaneous spiking activity of populations of neurons in the medial prefrontal cortex (mPFC) revealed accurate coexisting representations of both the transient sensory cues as well as the instantaneous speed of the animal during the delay period while the animal runs towards the TL. We looked for evidence of active executive control by dissecting the relationship between population activity and speed at different locations during the trial. In the middle of the delay period, as the mice ran towards the TL, the highest accuracy of speed decoders was achieved by shifting the speed signal backwards in time relative to the neurons, consistent speed functioning as a driving input, presumably necessary for state estimation. In contrast, right before the TL, when the mouse is slowing down, the highest accuracy was achieved shifting the speed signal forward in time with respect to the neurons, consistent with the neurons causing the slowing down response. We suggest that the temporal reversal between neural activity and behavior during the trial is a defining signature of executive control, which we demonstrate can be revealed in the mouse mPFC.

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Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.01/X44

Topic: H.01. Animal Cognition and Behavior

Support: CRC/Transregio 58

Title: Endocannabinoids modulate fear responses to predictable and unpredictable threat through CRH neurons in the extended amygdala network

Authors: *J. L. REMMES[1], M. D. LANGE[1], J. C. BARTSCH[1], F. REMMERS[2], B. LUTZ[2], H.-C. PAPE[1];
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Abstract: Phasic states of fear in response to a discrete threat can shift to a prolonged state of anxious apprehension, which is regarded as sustained fear. Sustained fear particularly occurs as a response to diffuse cues with unpredictable environmental contingencies. It has been shown that sustained fear is mediated via cannabinoid type 1 (CB1) receptors on distinct amygdala inputs to neurons in the anterolateral group of the bed nucleus of stria terminalis (alBNST). However, subpopulations of neurons driving the CB1 effect remain elusive. As previous studies on corticotropin-releasing hormone (CRH) suggest a critical role for CRH in modulating anxiety in humans and mice, we hypothesize that CRH-positive neurons exert their regulatory control over transitory states of fear via CB1-receptors.Thus, in a combined approach of retrograde tracing, optogenetic and electrophysiological studies, we investigated CB1 mediated synaptic interactions in CRH-positive neurons ex vivo. First results demonstrate that CRH is dominantly expressed in the projection from centrolateral amygdala (CeL) to alBNST, which in turn contains CB1-receptors. Additionally, we demonstrated that these CRH projection neurons are modulated by CB1-receptor activity in alBNST, suggesting a direct interaction of CRH and CB1-receptors in the extended amygdala network, which is gating states of fear. Next, we utilized a dedicated training protocol in mice, involving predictable or unpredictable threat, which allows distinction of phasic and sustained fear responses and is thus thought to adequately monitor the transition between fear and anxiety-like states in vivo. Using genetic loss-of-function and rescue approaches, and pharmacological intervention for CB1-receptors, we showed that CB1 activation on CRH-positive neurons in the alBNST drives the state of prolonged anxious apprehension, hereby providing evidence, that the observed interaction of CRH and CB1 is sufficient and necessary for sustained fear responses to unpredictable threat.

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Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.02/X45

Topic: H.01. Animal Cognition and Behavior

Support: CONACyT: CB-250870
DGAPA PAPIIT: IN212919

Title: Optogenetic stimulation of BLA-IC projections induces long-term avoidance memories

Authors: *A. HERNANDEZ-MATIAS[1], J. JAIMES-CABRERA[1], K. GUZMAN-RAMOS[2], F. BERMUDEZ-RATTONI[1], D. OSORIO-GOMEZ[1];
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Abstract: The insular cortex (IC) is a brain structure associated mainly with central integration of a wide variety of sensory signals, which includes gustative and visceral signals. Another equally important, but little-known function is related to cognitive processes. Thus, a large body of evidence indicates that IC is required for the proper formation and expression of different types of memory including recognition, addictive and avoidance memories. In the latter case, the projections from limbic structures to the IC, particularly from the basolateral amygdala (BLA), are critical for the establishment and retrieval of conditioned taste aversion (CTA). In this regard, we designed a series of experiments using an optogenetic approach to determine whether stimulation of the BLA-IC pathway might induce a conditioned taste avoidance in the absence of a gastric malaise agent. In addition, we evaluated whether these projections have a generalized participation in another type of avoidance memory. Male Wistar rats were bilaterally infused with the viral vector AAV5-CaMKII-hChR2(H134R)-eYFP into BLA, and fiber optic probes aiming to IC. The animals were optically stimulated through a double session of 10 minutes of blue light (473 nm) pulses at 20 Hz; stimulation occurred 15 minutes after saccharin consumption and 40 minutes following the first stimulation. The behavioral results showed a reduced saccharin consumption during long-term memory in the CTA test, this taste avoidance response was very similar to a naturally-induced CTA with lithium chloride (LiCl) injections in a control group. In addition, to assess if BLA-IC projections are involved in another avoidance memory task, we employed a Conditioned Place Avoidance (CPA) procedure. Briefly, rats received 3 acquisition trials in which blue light optical stimulation of BLA-IC projection was applied for 10 minutes when animals were confined in the preferred compartment of a conditioned place preference box; 30 minutes later, the animals were confined to the non-preferred compartment without optical stimulation. Compared to control animals, our results indicate that stimulation of the BLA-IC pathway significantly reduced the amount of time that animals spent in the compartment previously associated with optogenetic stimulation during long term memory test. All these data suggest that projections from BLA to IC can serve as a common neural substrate for aversive memories in spite of the different characteristics, contextual or gustatory, of the conditioned stimulus.

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Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.03/X46

Topic: H.01. Animal Cognition and Behavior

Support: DA041482
DA043184

Title: A role for distinct interpeduncular nucleus 5-HTergic circuits in fear memory consolidation and recall

Authors: *I.-J. YOU[1], L. LIU[2], A. SACINO[1], M. UCHIGASHIMA[3], K. FUTAI[1], A. R. TAPPER[1];
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Abstract: Recently, the interpeduncular nucleus (IPN) has been implicated as a critical neuroanatomical substrate for modulating fear memory. However, functional IPN afferent and efferent circuitry that contributes to fear memory is largely unknown. Interestingly, viral tracing studies indicate that the IPN receives projections from the medial raphe nucleus (MRN) and has serotonergic (5-HTergic) neuron soma in the caudal area of the nucleus, although 5-HTergic signaling in the IPN and the functional relevance of this MRN→IPN input has not been elucidated. Molecular and biophysical analysis of 5-HT receptors in the IPN indicated robust functional expression of 5-HT_{1A}, but not 5-HT_5 receptors. Double fluorescent in situ hybridization experiments revealed co-localization of the 5-HT_{1A} receptor with tryptophan hydroxylase2 and glutamic acid decarboxylase 67 transcripts suggesting 5-HTergic IPN inputs may innervate both 5-HTergic and GABAergic neurons in the rostral sub-nuclei of the IPN. To test the hypothesis that MRN→IPN 5-HTergic inputs contribute to fear memory via 5-HT_{1A} receptors, we blocked the 5-HT_{1A} receptor using a selective 5-HT_{1A} receptor antagonist in the IPN. Microinjection of the drug in the IPN prevented both contextual fear memory consolidation and recall. To test the contribution of MRN→IPN 5-HTergic inputs in fear memory, we expressed channelrhodopsin (ChR2) or halorhodopsin (NpHR) in the MRN 5-HTrgic neurons that project to the IPN using retrograde viral delivery in FEV-Cre mice. Optical stimulation of 5-HTergic terminals in the IPN enhanced only contextual fear memory consolidation; whereas, optical inhibition of MRN→IPN inputs decreased contextual fear memory consolidation. Next, we infected ChR2 and NpHR in IPN 5-HTergic neurons using anterograde viral delivery in FEV-Cre mice. Photostimulation of 5-HTergic neurons in the IPN selectively decreased contextual fear memory recall but not consolidation. These results reveal distinct 5-HTergic circuits within

the IPN control independent aspects of contextual memory.
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Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.04/Y1

Topic: H.01. Animal Cognition and Behavior

Title: Inhibiting the thalamic nucleus reuniens activity or protein degradation prior to memory reactivation prevents drug-induced reconsolidation impairment

Authors: *F. TROYNER[1], M. A. BICCA[2], L. J. BERTOGLIO[3];
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Abstract: The thalamic nucleus reuniens (NR) is known as a hub for processing memories which depend on the dorsal hippocampus (dH) and the medial prefrontal cortex (mPFC). However, its potential role in memory destabilization upon reactivation is still unknown. Due to its functional relationship with dH and mPFC, we hypothesize the NR function could influence memory destabilization. To address this question, we subjected adult male Wistar rats to a stereotaxic surgery aiming at the NR one week prior to contextual fear conditioning. The day after context-footshock pairing, the animals were infused with the vehicle, the GABA-A agonist muscimol or the proteasome inhibitor clasto-lactacystin beta-lactone (beta-lac) into the NR and 10 min later exposed to the conditioned context (reactivation session) for 5 min. The muscimol was used to temporarily inhibit the NR while the beta-lac was used to inhibit the local protein degradation associated with memory destabilization. Immediately after the reactivation session, animals were treated into the NR with anisomycin (a protein synthesis inhibitor) or systemically with clonidine (an alpha-2 adrenoceptor agonist). These two drugs have been shown to impair aversive memory reconsolidation. The next day, animals were again exposed to the paired context (Test A). All groups presented statistically similar freezing time during the reactivation session. During Test A, both vehicle-anisomycin and vehicle-clonidine groups presented statistically significant ($p \leq 0.05$) less freezing time than controls, confirming the drug-induced reconsolidation impairment in either case. In contrast, animals treated with muscimol or beta-lac before and anisomycin or clonidine after memory reactivation did not differ from respective control groups, indicating that silencing the activity in NR, or blocking its proteasome activity,

prevents drug-induced reconsolidation impairment. Altogether, present results suggest this thalamic subregion contributes to contextual fear memory destabilization.

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Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

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NK 72959
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Title: *In vivo* evidence of sharp wave-ripple associated dendritic Ca^{2+} signals in hippocampal interneurons

Authors: *Z. MEZRICZKY[1], G. JUHÁSZ[1,2], B. CHIOVINI[2], D. PÁLFI[1,2], L. JUDÁK[2], G. KATONA[2,1], B. RÓZSA[2,1];
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Abstract: Sharp wave-ripple complexes (SPW-Rs) have key role in the memory formation. These well defined local field potentials resulted by synchronized population activity of pyramidal cells and interneurons in hippocampus. SPW-R activities appear during slow wave sleep, consummatory behaviour and in awake immobility periods. Our previous work proved that not only the population activity participates in the formation of SPW-R-s, but also synaptically activated dendritic segments.These work was based only on *in vitro* measurements and the SPW-Rs associated dendritic signals had not been investigated *in vivo*.Here we measured the dendritic Ca^{2+} responses of parvalbumin containing interneurons simultaneously with local field potentials in CA1 region of the hippocampus. We developed new surgical methods in order to apply two-dimensional (2D) and three-dimensional (3D) two-photon random-access point scanning imaging techniques combined with ipsilateral multi-channel electrophysiology.We found different dendritic Ca^{2+} events associated with SPW-Rs in the hippocampus of awake animals. Our imaging system was able to detect dendritic Ca^{2+} spikes in hippocampal interneurons. Our results contribute to the better understanding of hippocampal coincidence detection and dendritic integration mechanisms of memory formation.

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Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

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Program #/Poster #: 082.06/Y3

Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI (19H04986(H.M.) 19H05225(K.M.), 18H05137(K.M.), 16H04656 (K.M.)),
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the Takeda Science Foundation (K.M.)
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Title: Evolution of fear memory representation in local and global circuits

Authors: *H. MIYAWAKI, K. MIZUSEKI;
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Abstract: Animals acquire memories through experiences, consolidate them during sleep, and retrieve them upon demands. The system consolidation of memory posits that newly acquired information are temporally deposited in the hippocampus as a labile form of memory trace and later transferred to other brain regions and stored as a long-lasting memory trace. Thus, it suggests that information representation would change during memory consolidation at least in the global circuit level. On the other hand, recent findings of engram cells proposed that same or very similar neuronal assemble within a brain region is active both during acquisition and retrieval of a memory. Thus, changeability and constancy in information representation are suggested in global and local circuit levels, respectively.
To seek how these discrepant views are incorporated, we recorded activities of hundreds of neurons in multiple brain regions simultaneously through the memory process. We used fear conditioning as a model system because timing of memory acquisition, consolidation, and retrievals are clearly separated. Accumulating evidence indicates fear memory involves the amygdala, ventral hippocampus and prefrontal cortex. Thus, we performed simultaneous large-scale electrophysiological recording in these brain regions of freely moving rats. We used electrical pulses applied through eyelid electrodes as unconditioned stimuli, which minimize shock artifacts and enable us to record neuronal activity through entire memory process including acquisition. To quantify fear and to score sleep states, we also recorded electrocardiogram, electromyogram of nuchal muscles, electrocorticogram of the olfactory bulbs,

and head acceleration. Behavioral sessions consist of baseline, conditioning, context-retention, cue-retention and extinction, and retention of extinction, each separated with 2.5-hour sleep sessions. The recording begun at a sleep session prior to baseline sessions and continued until a sleep session following retention of extinction session.
We examined how information of freezing conveyed by each spike changed across the behavioral sessions. Majority of cells did not change information content, but some neurons gained information (information gaining cells) and the others lost (information losing cells). Interestingly, fraction of information gaining/losing cells varied across brain regions. These results indicate that neuronal cording changes through memory process both in local and global levels. We will present how neuronal cording changes in single cells, local circuits, and global circuits, and how the cording evolves across sleep.

Disclosures: H. Miyawaki: None. **K. Mizuseki:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.07/Y4

Topic: H.01. Animal Cognition and Behavior

Support: European Commission M-GATE 765549

Title: Observing consolidation: Calcium imaging of long-term object memory traces in anterior cingulate cortex

Authors: *L. A. L. DESCAMPS[1], J. R. MAXEY[2], T. ROGERSON[2], M. J. SCHNITZER[2], C. G. KENTROS[1];
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Abstract: Lesions of the hippocampus (HPC) lead to profound anterograde amnesia, clearly implicating this structure in memory acquisition. However, memories formed prior to HPC lesion are largely spared, leading to the idea that memories are transferred out of the HPC for long term storage, a process called systems consolidation. While the precise anatomical basis for memory consolidation is unclear, the mouse anterior cingulate cortex (ACC) has been extensively studied as a substrate for remote (beyond 4 weeks) fear memory ([1]). Our prior work ([2]) demonstrated that the ACC also shows electrophysiological correlates of object memory on a similar timescale. Here, we investigated how these correlates form and stabilize to allow for the expression of remote object memory. Weible et al familiarized mice to two objects either once or for an entire week, and then returned the animals to the arena with one object removed (absent-object session). ACC neurons fired at object locations throughout the familiarization sessions, though these object correlates were initially unstable. Some of the cells

in the animals that had been trained for a week also fired at the location of the removed object. These absent-object correlates even persisted 30 days after the last time the animal had seen the objects, suggesting a long-term memory of the object/place association. However, Weible et al were not able to record from the same cells during both the object habituation and the absent-object sessions, therefore could not watch the dynamics of the process leading to object traces being consolidated. Here, we used a miniature head-mounted fluorescence microscope to perform neuronal calcium-imaging in freely moving transgenic mice expressing a calcium indicator in excitatory cortical neurons, to monitor the same population of cells from the first object habituation session to the absent-object task, but also throughout the consolidation period in between. This gives us the advantage to observe how ACC object correlates form, what the time course of their stabilization is, and how their consolidation is dependent upon initial repeated exposure.
[1] Frankland et al, Science 2004 [2] Weible et al, Journal of Neuroscience 2012

Disclosures: **L.A.L. Descamps:** None. **J.R. Maxey:** None. **T. Rogerson:** None. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inscopix Inc.. F. Consulting Fees (e.g., advisory boards); Inscopix Inc.. **C.G. Kentros:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.08/Y5

Topic: H.01. Animal Cognition and Behavior

Support: DGE1144152
DGE1745303

Title: Tracking large-scale neuronal populations in lateral visual cortex across cue-outcome association learning

Authors: *K. L. MCGUIRE[1,2], R. N. RAMESH[1,2], C. R. BURGESS[3,2], A. U. SUGDEN[2], J. D. ZAREMBA[2], M. L. ANDERMANN[1,2];
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Abstract: Rodent and primate lesion studies and human neuroimaging studies suggest that lateral visual association cortex is critical for linking stimuli with predicted outcomes. Previously, our lab has shown that pharmacological silencing of postrhinal cortex and surrounding regions of lateral visual association cortex in mice performing a Go-NoGo orientation discrimination task dramatically impairs their ability to selectively respond to

presentation of a stimulus associated with reward. In particular, mice continue to perform the task, but now respond equivalently to all three stimuli—including an unrewarded and a punished stimulus—suggesting an impairment of stimulus-outcome associations. While substantial progress has been made in understanding the effects of learning on visual circuits, very few studies have tracked cortical neurons throughout the course of sensory learning (e.g., Peters *et al*. 2014). We used two-photon calcium imaging to track visual responses of the same large set of hundreds of neurons in behaving mice across dozens of sessions spanning the entire training process. We aligned responses of the same neurons over months of imaging (714-2285 neurons per mouse across 5 mice; 12-53 imaging days per mouse, median 30) starting in naïve mice, during gradual learning of a three-orientation discrimination task, and following a change in cue-outcome contingencies. Preliminary analyses suggest that distinct ensembles of neurons respond stably to specific visual stimuli throughout the course of training, while other ensembles change their response properties over the course of learning. Using large-scale population analyses, including dimensionality reduction techniques (e.g. tensor component analysis), we are beginning to elucidate the distinct computations of ensembles of neurons in lateral visual association cortex in an unbiased manner across stages of association learning.

Disclosures: K.L. McGuire: None. **R.N. Ramesh:** None. **C.R. Burgess:** None. **A.U. Sugden:** None. **M.L. Andermann:** None. **J.D. Zaremba:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: Harvard University Mind Brain Behavior Interfaculty Initiative
NIH T32 5T32DK007516
NIH New Innovator Award DP2 DK105570
R01 DK109930
McKnight Scholar Award
Pew Scholar Award
Smith Family Foundation Award

Title: Associative learning in mouse visual association cortical circuits

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Abstract: We are constantly adapting to the world around us, learning from experiences to better predict future states of the world. Rapid learning of initial experiences is refined through the active process of memory recall and consolidation, by which memory traces are stabilized and transformed. We examined the activity of large ensembles of neurons in mouse visual association cortex both while mice are presented with visual stimuli and then also at later times either between trials or in entirely dark sessions absent of any visual input. During these sessions, we identified patterns of activity similar to what is observed during stimulus presentation, but in the absence of any explicit visual stimulation - events termed stimulus reactivations. By tracking the content of these reactivations throughout learning of a visual Go-NoGo cue-outcome association task, we can begin to assess circuit-level processes involved in the binding of visual cues to rewarding and aversive outcomes. We observed cue reactivations throughout the inter-trial interval and subsequent dark sessions. In addition, we observed predictive representations of reward in visual association cortex that evolve with learning. The joint offline reactivation of representations of cues and subsequent outcomes may be critical to the associative binding of these temporally non-overlapping events. By utilizing two-color two-photon imaging, we are beginning to investigate the interaction between multiple information streams and to monitor downstream consolidation-dependent circuit changes.

Disclosures: J.D. Zaremba: None. **A.U. Sugden:** None. **K.L. McGuire:** None. **E.H. Thompson:** None. **K.K. Lensjø:** None. **O. Alturkistani:** None. **M.L. Andermann:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.10/Y7

Topic: H.01. Animal Cognition and Behavior

Title: The Locus Coeruleus activity during hippocampal-cortical communication

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Abstract: The brain stem noradrenergic nucleus Locus Coeruleus (LC) is the primary source of norepinephrine (NE) in the forebrain. The LC-NE system plays a critical role in regulating arousal state, it mediates many visceral responses and also involved in a variety of cognitive functions including learning and memory. Enhanced tonic firing of LC neurons during sleep is associated with a decrease in the occurrence of hippocampal ripples and sleep spindles; both oscillatory events have been suggested to mediate the hippocampal-cortical communication

underlying systems-level consolidation. Experimentally induced ripple-triggered phasic LC activation during post-learning sleep causes memory deficit, possibly due to interference with cellular- and systems-level memory consolidation mechanisms. At present, the involvement of LC-NE system in the hippocampal-cortical information transfer remains largely unexplored. In the present study, we examined temporal coupling between the LC spiking activity, hippocampal ripples, and sleep spindles. The multiunit (MUA) recordings were obtained from the LC simultaneously with the local field potentials (LFPs) from the CA1 subfield of dorsal hippocampus and the prefrontal cortex in rats during spontaneous behavior. In general, the LC-MUA was suppressed around ripples and elevated around sleep spindles. Systematic fluctuations of LC-MUA around ripples occurred at faster (~500 ms) and slower (~10 sec) temporal scales. Specifically, a gradual reduction of LC tonic firing occurred ~10 sec preceding ripple oscillation and an additional sharp transient decrease of LC-MUA was observed ~ 1 sec prior the ripple onset. Notably, the most pronounced LC modulation at slower time scale occurred before ripples co-occurring with sleep spindles. The degree of LC activity modulation at faster time scale was comparable among awake or sleep ripples. Fast LC-MUA modulation was essentially absent around ripples coupled with sleep spindles. Our results suggest that the LC activity suppression may facilitate transition to a specific brain state that is favorable for 'off-line' inter-regional information transfer.

Disclosures: **M. Yang:** None. **N.K. Logothetis:** None. **S.J. Sara:** None. **O. Eschenko:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.11/Y8

Topic: H.01. Animal Cognition and Behavior

Support: ONR (N00014-16-1-2829)
Lifelong Learning Machines program from DARPA/MTO (HR0011-18-2-0021)

Title: Avoiding catastrophic forgetting by selective memory reactivation during slow wave sleep

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[1]Med., [2]Dept. of Med., UCSD, La Jolla, CA; [3]Univ. of California San Diego, San Diego, CA;
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Abstract: Systems consolidation theory posits that the interaction between hippocampal and neocortical networks is critical for the long-term storage and conscious retrieval of memories. Accumulating evidence points to the important role of sleep in coordination of the hippocampal and neocortical network dynamics to consolidate recent memories. Hippocampal indexing may provide a route by which this interaction leads to formation and strengthening of long-term

neocortical memory representations. The exact mechanisms behind memory consolidation during sleep remain to be understood. In this study, we explored how selective memory (re)activation during slow-wave (N3) sleep allows the formation of new memory traces within a previously trained thalamocortical network without catastrophic forgetting of the old memories. We used a computational model of the thalamocortical network which is capable of transitioning between periods of wake and N3 sleep and implements spike-timing dependent plasticity on excitatory AMPA connections between cortical neurons to allow for synaptic weight changes associated with new learning and consolidation. Memory traces were represented by the spatio-temporal patterns of ordered spiking among selected groups of the cortical neurons. Training the network on a sequence S1 during awake state resulted in selected increase of synaptic weights and performance on a sequence completion task. When a "new" memory S1*, that overlaps and competes with S1 was trained subsequently in awake, it resulted in degradation of S1. Next, to implement basic principles of the complimentary memory system, we delivered S1* during N3 sleep to simulate effect of hippocampal ripples. We found that when S1* was delivered near the transition point from cortical Down-to-Up states, replay of S1 and play of S1* occurred simultaneously within the cortical network leading to performance improvement for both memories. In this way, the network was able to store two overlapping competing memory sequences through the selective activation of S1* to form a new memory trace and reactivation of S1 to avoid forgetting of the old memory. When Up/Down state structure of the N3 sleep was replaced by a continual Up state, the old memory S1 was replayed and improved but the new S1* memory failed to form. Together, these predict that (a) precisely timed hippocampal input can enable embedding of recent memories to the cortical network during N3 sleep and (b) oscillatory structure of the Up/Down state transitions during N3 sleep is critical for this process. The study provides new insights into the underlying mechanism by which hippocampal indexing aids in the consolidation of recent memories.

Disclosures: E. Delanois: None. **O.C. Gonzalez:** None. **G.P. Krishnan:** None. **M.V. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.12/Y9

Topic: H.01. Animal Cognition and Behavior

Support: Lifelong Learning Machines program from DARPA/MTO (HR0011-18-2-0021)
ONR (N00014-16-1-2829)
NIH (R01MH117155)

Title: Competitive inhibition and heterosynaptic plasticity prevent memory interference through pattern separation of overlapping memories during wake and sleep

Authors: *J. C. DOMINGUEZ, Jr[1], R. GOLDEN[2], G. P. KRISHNAN[2], M. V. BAZHENOV[2]; [1]Dept. of Cognitive Sci., [2]Dept. of Med., UCSD, La Jolla, CA

Abstract: Humans and animals are generally able to discriminate various natural stimuli from one another without much effort and can even learn to discriminate between incredibly similar artificial stimuli. Moreover, a large body of literature has shown that discriminability can be further improved from consolidation during sleep following learning. Previous work in our lab has suggested that slow-wave sleep can accomplish this through a complex reorganization of the synaptic connectivity matrix that maximizes separation between representations of the interfering memories. Nevertheless, if the stimuli are too similar, the brain might not be able to learn to discriminate due to the strong interference. It remains unclear which network properties are responsible for this threshold and how the brain can control it. There has recently been evidence suggesting that increasing the amount of inhibition in the network can improve discriminability, but the quantitative relationship and mechanism are still unknown. In this new study, we developed a biophysically-realistic thalamocortical network model where we could train multiple memories with different degrees of interference to systematically explore how discriminability varies with stimulus similarity and which network properties may affect it. We varied parameters controlling the amount and effects of inhibition and found that we could indeed control the value of the similarity threshold in the network. Additionally, we found that competitive inhibition had a greater influence over this threshold value compared to feedforward or feedback inhibition. Finally, we found that while slow-wave sleep could cause an increase in performance for a broad range of discriminable stimuli, this effect was more robust if heterosynaptic plasticity was included in the network model in addition to spike-timing-dependent plasticity. Without this mechanism, the network tends to over-generalize by recruiting task-irrelevant neurons into the memory engram, thus reducing the signal-to-noise ratio for decoding and reducing network capacity for further learning. The study predicts that competitive inhibition can improve discriminability during learning and heterosynaptic plasticity can reduce memory interference during sleep.

Disclosures: J.C. Dominguez: None. **R. Golden:** None. **G.P. Krishnan:** None. **M.V. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.13/Y10

Topic: H.01. Animal Cognition and Behavior

Support: DARPA/MTO HR0011-18-2-0021
ONR N00014-16-1-2829
NSF IIS-1724405

Title: Slow-wave sleep helps reduce interference of the overlapping memory traces

Authors: *Y. SOKOLOV, G. P. KRISHNAN, M. V. BAZHENOV;
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Abstract: The human and animal brains are able to acquire new knowledge every day. To effectively store new memories, the brain must utilize a nontrivial encoding mechanism so that each memory is described by a unique ensemble of interconnected neurons, which would guarantee a robust memory recall. However, while animal and human brains have extremely large number of neurons and synapses, the input space is limited and therefore there is a risk of overlap between ensembles of neurons representing different memories. To understand how the brain is capable of overcoming this problem and how the brain separates representations of the potentially interfering memory traces, we considered a computational model of the thalamocortical network with random synaptic connectivity. Spike timing dependent synaptic plasticity (STDP) was implemented along with heterosynaptic scaling mechanisms to account for memory training and consolidation. Different memories were encoded within selected populations of the cortical pyramidal neurons during sequential training in awake-like state. Trained network was able to complete individual patterns without overlap when presented with only partial input. However, when multiple patterns with partial overlap were trained, there was a reduction in the ability of the network to perform pattern-completion on the newly acquired overlapping memories. When a period of a slow-wave sleep-like activity was applied following initial training, the network was able to complete overlapping patterns. While preserving overlap of the memory representations, slow-wave sleep dynamics separated the patterns by reorganizing the connectivity profile among overlapping memories. Our study suggests that sleep helps to separate the overlapping memories and to reduce the interference without explicit removal of the overlapping neuronal ensembles from the memory patterns.

Disclosures: Y. Sokolov: None. **G.P. Krishnan:** None. **M.V. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.14/Y11

Topic: H.01. Animal Cognition and Behavior

Support: ONR (N00014-16-1-2829)
Lifelong Learning Machines program from DARPA/MTO (HR0011-18-2-0021)

National Institute of Health (R01MH117155)
National Science Foundation (IIS-1724405)

Title: Neural mechanisms of reactivation during slow-wave sleep

Authors: *G. P. KRISHNAN[1], O. C. GONZALEZ[3], R. RAMYAA[4], M. V. BAZHENOV[2]; [1]Univ. of California San Diego, San Diego, CA; [2]Dept. of Med., Univ. of California San Diego, La Jolla, CA; [3]Dept. of Med., UCSD, La Jolla, CA; [4]New Mexico Tech., Socorro, NM

Abstract: Spontaneously occurring neural activity during sleep has been observed to closely relate to past experiences. Memory reactivation has been hypothesized to be critical for memory consolidation. However, the neural mechanisms behind the memory reactivation and replay during sleep are not well understood. In this new study, we applied a computational model of the thalamocortical network with spike-timing dependent synaptic plasticity to examine the various intrinsic, synaptic and connectivity factors that determine memory reactivation during sleep. We trained the network in the 'awake' state, when neuromodulator levels (acetylcholine, norepinephrine and histamine) were high, by activating a pattern represented by an ordered firing among the groups of cortical neurons. The training led to increase in synaptic weights in the connections that were aligned with the learned sequence. NREM sleep like state with slow oscillations was then spontaneously generated when the levels of the neuromodulators were reduced. Memory reactivation during sleep was measured based on the spike timing of the neurons within and between trained groups. Average reactivation score during sleep was obtained as the Euclidian distance between all the reactivated sequences and the 'ideal' trained sequence. We found that the strength of excitatory connections between cortical pyramidal neurons and potassium leak currents were the main factors affecting the level of memory reactivation during NREM sleep. An increase of the AMPA current led to increase in memory reactivation, while the increase of the leak currents reduced reactivation. The change in synaptic connections followed sleep reactivation was higher when the excitatory connections were increased. We developed a simplified probabilistic neuron model that explains the effect of excitatory synaptic strength on memory reactivation during NREM sleep. Overall, our results suggest that the neuromodulatory changes during sleep determine recurrent excitatory strength and excitability of neurons to bias the activity during sleep and to facilitate memory reactivation of past experiences.

Disclosures: G.P. Krishnan: None. **O.C. Gonzalez:** None. **R. Ramyaa:** None. **M.V. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.15/Y12

Topic: H.01. Animal Cognition and Behavior

Support: ONR (N00014-16-1-2829)
and Lifelong Learning Machines program from DARPA/MTO (HR0011-18-2-0021)

Title: Catastrophic forgetting and continual learning in a multi-layer spiking network with reward-modulated spike-timing-dependent plasticity

Authors: *R. G. GOLDEN[1], E. DELANOIS[2], M. V. BAZHENOV[1];
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Abstract: Biologically inspired neural networks of spiking neurons combined with realistic homeostatic mechanisms along with reward-modulated spike-timing-dependent plasticity (R-STDP) have been shown to perform well on complex foraging tasks [Skorheim at al. PLoS One 2014; Sanda et al. PLoS Comp Bio 2017]. Here we expand upon these studies to investigate mechanisms of catastrophic forgetting (CF), a phenomenon where a network forgets a previously learned memory while acquiring a new one. CF is common across artificial neuronal networks, however, humans and animals do not usually experience CF and are capable of continual learning. In this study, the network policy governs the movement of an agent through a virtual environment which can be equipped with two distinct foraging tasks, each requiring discrimination between different rewarded and non-rewarded food particles. When the network was trained on either task individually, a high level of performance was achieved. However, when a second task was trained subsequently after the 1st one, only the 2nd task was learned and performance on the original task reduced to the baseline, exemplifying CF. It is believed that sleep can prevent CF in biological system by replaying recent memories along with the old relevant memories. To mimic replay in the network, we applied an interleaved training paradigm, where the network was trained on examples from both tasks in a rapidly alternating fashion. Interleaved training was able to recover performance on the first task while maintaining performance on the most recently learned task. A support vector machine was trained to classify synaptic weight matrices and a clear decision boundary was observable between synaptic weight configurations representing each of two tasks. Interleaved training consistently brought the network to a state significantly closer to the decision boundary, showing interleaved training hybridized the weight states between the original and newly learned task, yielding an optimal synaptic structure for multi-task performance. To test if a noise driven replay combined with unsupervised plasticity could simulate biological sleep, the network was trained to a sub-maximum performance before artificially increasing the top quartile of synaptic weights. This resulted in a substantial increase in performance, indicating that early on in training a synaptic structure exists that noisy replay could potentially exploit and enhance. This study reveals properties of the synaptic weight dynamics behind CF in a spiking network and predicts that noisy replay may potentially prevent CF.

Disclosures: R.G. Golden: None. **E. Delanois:** None. **M.V. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 082.16/Y13

Topic: H.01. Animal Cognition and Behavior

Support: ONR (N00014-16-1-2829)
Lifelong Learning Machines program from DARPA/MTO (HR0011-18-2-0021)

Title: Simulated sleep helps to generalize knowledge in a spiking network trained with spike-timing dependent plasticity

Authors: *T. TADROS[1,2], G. P. KRISHNAN[2], M. V. BAZHENOV[2];
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Abstract: The current state of artificial neural networks suffers from a generalization problem – they excel at representing data seen so far at the expense of representing data not explicitly learned. This problem results in the need for expansive training sets that capture the variability in the task at hand which may not always exist. In the mammalian brain, evidence suggests that sleep promotes generalization of learned examples. Replay during sleep of different versions of learned information may enable the brain to form a much more generalized representation of the data. To address the validity of this hypothesis, we utilized a spiking neural network trained with spike-timing dependent plasticity (STDP) previously proposed to perform digit classification on the MNIST dataset. While this network can learn the task, it only reaches high levels of performance (>90%) after training on more than 100,000 images. We took a partially trained network (between 20 and 80% of the full training image set) and applied a sleep-like phase after the learning phase. During the simulated sleep, we modified the intrinsic and synaptic currents to mimic changes in neuromodulator levels, while presenting noisy Poisson input based on the statistics of the MNIST input. We observed an improvement in performance after sleep compared to baseline (before sleep) across different levels of baseline training. This result was most pronounced for the lower levels of baseline training (<50% of full training set) where performance increased from a mean of ~40% to ~55% after sleep. We also found that sleep increased the network's ability to classify digits with various types of noise compared to before sleep, indicating an ability to generalize beyond the training set. Next, we analyzed how changes in the spiking threshold of the individual neurons and synaptic weights affected performance. Initially, the neuronal threshold changes induced by sleep resulted in the greatest performance increase. We also observed the same level of performance improvement arising solely from STDP changes, when the thresholds were fixed and inhibition was reduced during sleep to increase excitability. Analysis of the receptive fields of the neurons revealed that neurons with

task-specific receptive fields experienced very little change. However, neurons with noisy, non-task-specific receptive fields experienced synaptic depotentiation, suggesting that sleep improves generalization by pruning noisy synapses. Ultimately, these results suggest that sleep may play an important role in solving the problem of generalization when large datasets are not available.

Disclosures: **T. Tadros:** None. **G.P. Krishnan:** None. **M.V. Bazhenov:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.01/Y14

Topic: H.01. Animal Cognition and Behavior

Support: NIH-CRCNS 5U01MH115746-02

Title: Network dynamics of cortico-entorhinal-hippocampal coupling during slow wave sleep: Experiment and theory

Authors: *K. CHOUDHARY[1,2,3,4], S. BERBERICH[5], J. M. MCFARLAND[6], T. T. HAHN[7], M. R. MEHTA[1,8,2,3,4];
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Abstract: During Slow Wave Sleep (SWS), the neocortex undergoes synchronous transitions between periods of tonic-firing and periods of quiescence; these are known as Up-Down States (UDS) [1]. Building on previous works [2-4], we find that the membrane potential of individual neurons in various brain regions (LEC, Frontal, Prefrontal, Parietal) oscillates in synchrony with simultaneously measured UDS in cortical LFP. On the other hand, the membrane potential of layer III neurons of the Medial Entorhinal Cortex (MEC) shows both spontaneous persistent activity and inactivity in relation to cortical Down and Up states, respectively. We propose a mean field attractor model, consisting of coupled networks of continuous excitatory and inhibitory units, to understand this differential cortico-entorhinal oscillatory dynamics. The model predicts a relationship between the strength of excitation in the neocortical drive to the MEC and the probability that the MEC network will decouple from the neocortical one, thereby exhibiting persistent activity/inactivity. Spectral analysis of the *in vivo* data confirms this relationship; particularly, we find that the amount of high frequency power (gamma band: 35-90 Hz) found in the cortical LFP is predictive of whether an MEC III neuron will exhibit persistence

during UDS. The model network is extended to include the hippocampus, and model parameters are fit to the *in vivo* data, which shows that Hippocampal CA1 activity during SWS is weakly but significantly modulated by MECIII persistence. The confluence of theoretical and experimental results suggests interesting differences between the MEC Layer III network and the network of other brain regions, and helps clarify the nature of cortico-entorhinal-hippocampal interaction, which is thought to be involved in working memory, the learning of behavioral sequences, and memory consolidation during sleep [5]. 1. Steriade, et. al. *J. Neuro.* (1993) 2. Hahn, et. al. *Nat Neuro (2012)* 3. Berberich, et. al. *SfN Abstract (2015)* 4. Choudhary et. al. *SfN Abstract (2018)* 5. Mehta et. al. *Nat Neuro (2007)*

Disclosures: K. Choudhary: None. **S. Berberich:** None. **J.M. McFarland:** None. **T.T. Hahn:** None. **M.R. Mehta:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.02/Y15

Topic: H.01. Animal Cognition and Behavior

Title: Hippocampal anchor fields

Authors: *M. SHAHI[1], S. DHINGRA[2], R. SANDLER[2], R. RIOS[2], C. VUONG[4], L. ACHARYA[5], M. R. MEHTA[3];
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Abstract: Abstract: Rodent hippocampal neurons show allocentric spatial selectivity. Recent studies show that some of them also show selectivity to head-direction, measured with respect to a coordinate frame at infinity. However, these are all local measures whereas spatial perception requires spatial information about disparate locations but such a signal does not exist. Here we show that nearly all CA1 place cells showed significant head-direction tuning with respect to a specific anchor point localized within the maze, termed hippocampal ‘anchor field’. There were no salient stimuli or rewards at the anchor points. The anchor field of a neuron was more likely to be near its place field, but almost never fully overlapping, indicating non-local mechanisms. Neurons with similar place fields often had entirely different anchor fields, showing a simultaneous representation of multiple anchor positions at the same spatial location. Most neurons also showed significant direction selectivity with respect to an anchor point at infinity. Thus the activity of place fields is described by a six-dimensional space. The degrees of spatial,

head directional and anchor selectivity of neurons were significantly correlated, implicating common underlying mechanisms. Visual cue manipulations had comparable and substantial effects on these six-dimensions of spatial selectivity. Thus, anchor tuning demonstrates a novel type of selectivity of hippocampal place cells, which could play a crucial role in binding different spatial locations to form a coherent, vectorial, six-dimensional representation of space

Disclosures: **M. Shahi:** None. **S. Dhingra:** None. **R. Sandler:** None. **R. Rios:** None. **C. Vuong:** None. **L. Acharya:** None. **M.R. Mehta:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.03/Y16

Topic: H.01. Animal Cognition and Behavior

Title: Differential modulation of hippocampal pyramidal cells and interneurons by the eta rhythm

Authors: *K. SAFARYAN[1], M. R. MEHTA[1,2,3,4];
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Abstract: Hippocampal theta oscillations in rodents profoundly impact neural synchrony, spatial coding, synaptic plasticity and learning (Green, Arduini, 1954; Winson, 1978; Buzsáki, 2002). However, the sensory mechanisms governing theta, which may underlie theta differences across species (Watrous et al., 2013; Goyal, A. et al., 2018), remain to be fully understood (Bland et al., 2006, 2007; Moore et al., 2013; Kleinfeld, Deschenes, Ulanovsky, 2016). We hypothesize that the nature of multisensory inputs is a crucial factor in hippocampal rhythmogenesis. Hence, we compared the rodent's hippocampal slow oscillations in the multisensory-rich real world (RW) and in a body-fixed, visual virtual reality (VR) (Ravassard et al. 2013). The rhythmicity of the hippocampal ~8 Hz theta activity was significantly enhanced in VR compared to RW. This was accompanied by the emergence of a ~4 Hz oscillation, termed the eta rhythm, seen in the local field potential (LFP) in VR, but not in RW (Safaryan, Mehta, 2017, 2018). Similar to theta, eta band amplitude increased with running speed in VR, but not in RW. However, contrary to theta, eta amplitude was highest in the CA1 cell layer, suggesting underlying intra-CA1 mechanisms. Consistently, putative CA1 interneurons, but not pyramidal neurons, showed substantially more eta modulation in VR than in RW. These results elucidate the multisensory mechanisms of hippocampal rhythms and the surprising effects of VR on neural ensemble dynamics bridging the gap in understanding differences in hippocampal theta oscillations across species.

Disclosures: **K. Safaryan:** None. **M.R. Mehta:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.04/Y17

Topic: H.01. Animal Cognition and Behavior

Support: NIH-CRCNS 5U01MH115746-02
NSF Eager
NSF Career
W. M. Keck Foundation

Title: Representations for real world space during virtual reality navigation in the rat hippocampus

Authors: *C. PURANDARE[1,2,3,4], K. CHOUDHARY[5,2,3,4], M. R. MEHTA[6,3,2,4];
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Abstract: During unconstrained real world (RW) exploration, rodent hippocampal activity shows robust spatial selectivity, which is hypothesized to be governed by distal visual locomotion cues, along with contributions from other sensory-motor cues. To dissect the contributions of these different variables on hippocampal spatial selectivity, we have recently developed a virtual reality apparatus. Here, the rat runs on a spherical treadmill that floated freely on an acoustically quiet air cushion, to receive liquid rewards through a tube in front of the rat. Our VR system employs a hinged harness that gently body-fixes the rats but allows for head movements around body. The harness, reward tube and VR chassis constitute a novel, constrained real world (c-RW) where the rat's legs and head can move freely but his body does not. Movement of his legs cause a rotation of the spherical treadmill that induces a change in the virtual scene, i.e. virtual movement, without any significant change in the rat's position in the c-RW position. In previous studies we found that during random foraging in VR, spatial selectivity is markedly reduced while directional modulation was comparable to RW [1, 2]. Here, we investigated the selectivity in c-RW while the rat performed two dimensional navigational tasks in the VR space. We find that a substantial number of neurons showed selectivity to the rat's head angle in the c-RW environment, in the absence of task demands in the c-RW frame and in the absence of active navigation driven changes in c-RW position (owing to body restriction). Our results suggest that hippocampal neurons can simultaneously maintain representations of

real and virtual environments and have important implications for the growing use of virtual reality for scientific, and commercial uses. 1. Aghajan et al, (2015), Nature Neuroscience volume 18 2. Acharya et al, (2016), Cell 164, 197-207

Disclosures: C. Purandare: None. **K. Choudhary:** None. **M.R. Mehta:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.05/Y18

Topic: H.01. Animal Cognition and Behavior

Support: NIH
W. M. Keck Foundation
NSF/CRCNS

Title: Rapid reorganization of hippocampal episodic-distance and allocentric-direction maps during a virtual navigation task

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Abstract: A cornerstone of hippocampal function is the Hebbian synaptic plasticity or the NMDAR-dependent plasticity, or STDP. While *in vitro* studies, computational models and *in vivo* experiments in one dimension provide ample support for this idea, the link between Hebbian plasticity, the emergent neural dynamics and behavioral performance has remained elusive. On a behavioral level, the hippocampus is implicated in episodic memory and allocentric spatial navigation. However, it is unclear if both episodic and allocentric variables are simultaneously represented in rodent hippocampal neurons, and what their relationship is with navigational performance. We recorded single units from dorsal CA1 of the hippocampus while rats executed a two-dimensional virtual navigation task based on the classic Morris water maze task. Although some neurons showed significant allocentric spatial selectivity, this was significantly impaired compared to that in typical real world tasks, despite excellent navigational performance. Instead, the majority of cells coded for episodic distance traveled. Cells also coded for the rat's allocentric head angle within the virtual space. Often, the same cells encoded both episodic and allocentric variables. The degree of tuning and the ensemble firing rate were correlated with performance across and within behavioral sessions. Consistent with computational models of the associative Hebbian learning, behaviorally relevant variables were overrepresented by neural responses. Further, consistent with models of navigation via temporally asymmetric Hebbian plasticity, performance improved significantly within each session, most neurons increased their

firing rates, and showed a large predictive shift in their episodic distance fields within a session. These findings demonstrate that hippocampal neurons can simultaneously exhibit task- and experience-dependent tuning to episodic and allocentric variables that could mediate navigation and that these emergent dynamics may be mediated by Hebbian synaptic plasticity.

Disclosures: **M.R. Mehta:** None. **J.J. Moore:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.06/Y19

Topic: H.01. Animal Cognition and Behavior

Support: NIH
W. M. Keck foundation
NSF CAREER
NSF EAGER
NSF

Title: Comparison of different basis functions in decoding hippocampal spatial maps

Authors: *S. SRINIVASAVARADHAN[1], Y. H. EZZELDIN[2], C. FRAGOULI[2], S. DIGGAVI[2], M. R. MEHTA[3];
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[3]Departments of: Physics & Astronomy, Neurology, Neurobio., Univ. of California at Los Angeles (UCLA), Los Angeles, CA

Abstract: The spatial selectivity of rodent CA1 pyramidal neurons in a two-dimensional space has been well-documented in the literature. For accurate population-decoding of position from neural measurements, a reliable estimation of the place field tuning function is required. For such purposes, machine learning paradigms such as the well-established *generalized linear model (GLM)* are often used to estimate the spatial tuning of a neuron from measured positional and neural data. An integral part of the GLM characterization of place field(s) is the representation of the position in terms of basis functions. Currently, the state of the art for open two-dimensional spaces, is to use *Zernike polynomial basis functions*[1]. In this work, we propose an alternative basis representation for the position using *Gaussian radial basis functions (GRB)* for which we observe favorable properties as compared to Zernike polynomial basis. To illustrate these properties, we fit GLM models for CA1 pyramidal neurons measured from a rodent foraging on a 2m circular platform with different distal visual cues. From the fitted models, we observe the following:
a. A GRB model can more naturally capture the shape of the firing rate maps of place cells,

especially for place cells having multiple disjoint place fields.
b. In the two-dimensional maze, the rat has not visited all possible positions (particularly towards the edge of the maze). For these un-visited positions, GRB provides a more stable representation of the tuning function.
c. Population-decoding with GLM models using GRB functions resulted in higher estimation accuracy of the rat position.
1Acharya, Lavanya, et al. "Causal influence of visual cues on hippocampal directional selectivity." Cell 164.1-2 (2016): 197-207.

Disclosures: S. Srinivasavaradhan: None. **Y.H. Ezzeldin:** None. **C. Fragouli:** 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.; NSF. **S. Diggavi:** 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.; NSF. **M.R. Mehta:** 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, W. M. Keck foundation, NSF EAGER, NSF CAREER.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.07/Y20

Topic: H.01. Animal Cognition and Behavior

Support: NSF Career Award 1565410 (IAM)

Title: Navigational affordances influence the use of geometric strategies in blind and sighted mice

Authors: *M. C. GARZA[1], T. I. ERESANARA[1], J. B. JULIAN[2], I. A. MUZZIO[1];
[1]Biol., Univ. of Texas at San Antonio, San Antonio, TX; [2]Kavli Inst. for Systems Neurosci., NTNU, Trondheim, Norway

Abstract: Spatial navigation requires the integration of internal and external cues. However, when navigators become lost or disoriented, internal cues are unreliable. In order to reorient, or regain one’s bearings, subjects must rely exclusively on the external geometry, e.g., the shape of the external layout. However, it is unknown how animals extract elements from the environment to navigate using geometric strategies. Moreover, since sighted navigators can use vision to rapidly process objects and background, while blind subjects must rely on haptic perception to

navigate, it is unclear if the reliance on geometry for reorientation is primarily observed in sighted animals. Our hypothesis was that both sighted and blind animals use the affordances of the traversable space to implement geometric strategies during reorientation. We tested this idea in three experiments. First, we trained mice to retrieve a hidden reward in an elevated rectangular platform (real cliff), or on a circular Plexi Glas platform containing a rectangular surface texture on it (artificial cliff). We found that both sighted and blind mice reoriented using geometric strategies only in the real cliff, suggesting that spatial affordances affect this approach. To further clarify if the presence of 3D edges also played a role in the ability of animals to reorient in the real cliff condition, we tested mice in an artificial cliff with an elevated rectangular platform where animals could step up or down to traverse the circular platform. Our results show that only blind animals were able to reorient successfully in this condition, highlighting the importance of 3D edges for haptic perception. Finally, we examined if the saliency of 3D boundaries affected the use of geometric strategies by testing animals in the artificial cliff surrounded by a large rectangular enclosure. We found that animals in this condition performed at random, confirming that space affordances are critical for the use of geometric strategies.

Disclosures: **M.C. Garza:** A. Employment/Salary (full or part-time):; University of Texas at San Antonio. **T.I. Eresanara:** A. Employment/Salary (full or part-time):; University of Texas at San Antonio. **J.B. Julian:** None. **I.A. Muzzio:** A. Employment/Salary (full or part-time):; University of Texas at San Antonio. 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.; NSF CAREER Award 1565410 (IAM).

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.08/Y21

Topic: H.01. Animal Cognition and Behavior

Support: NSF Career Award 1565410 to IAM

Title: Role of the retrosplenial cortex in spatial reorientation

Authors: *C. M. GAGLIARDI[1], M. E. NORMANDIN[2], J. H. VASQUEZ[2], N. PUNJAALA[2], I. A. MUZZIO[3];
[1]Univ. of Texas At San Antonio, San Antonio, TX; [2]The Univ. of Texas at San Antonio, San Antonio, TX; [3]Biol., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Reorientation, the process by which lost navigators regain their bearings, is fundamental for navigation. Under oriented conditions, navigators use both external and internal

cues to keep track of their bearings. However, during disorientation the internal sense of direction is unreliable and lost navigators must exclusively rely on external cues to reorient. Studies have shown that disoriented subjects use the geometry of the environment to reorient, despite directionally-informative featural cues (*i.e.*, textures, colors, odors, small moveable objects), which suggest that reorientation requires a cognitive, encapsulated geometric module. Here, we investigated the role of the retrosplenial cortex (RSC) in reorientation. The RSC has been shown to code the shape of environmental layouts and contains head-direction cells, which provide a compass on the horizontal plane. Therefore, we hypothesized that the RSC provides the directional signal that is necessary for reorientation. To address this idea, we first used chemogenetic silencing of the RSC while animals performed a classical reorientation paradigm. In this task, disoriented mice sought a reward hidden in one out of four cups placed near the corners of a rectangular environment containing a polarizing visual cue. We found that silencing the RSC impaired reorientation when mice used geometric strategies to solve the task. However, this manipulation had no effect in over-trained animals that solved the task by incorporating featural strategies (polarizing visual cue). Second, we recorded single-units in the RSC as mice performed the reorientation task. We found that retrosplenial cells with both location and head direction properties anchored their activity to environmental geometry. We are currently recording calcium activity in freely moving mice during reorientation to determine population dynamics in different types of RSC cells. These results will be critical to understand the role of the RSC in reorientation and will help to elucidate how different aspects of the environment control single cell activity in this region.

Disclosures: C.M. Gagliardi: None. **M.E. Normandin:** None. **J.H. Vasquez:** None. **N. Punjaala:** None. **I.A. Muzzio:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.09/Y22

Topic: H.01. Animal Cognition and Behavior

Support: University of Texas McGovern Medical School at Houston startup funds

Title: Brain waves under a computational microscope - New structures, new perspectives

Authors: *Y. A. D. DABAGHIAN;
Neurol., Univ. of Texas at Houston, McGovern Med. S, Houston, TX

Abstract: Extracellular Local Field Potentials (LFP) modulate neuronal activity at all levels: from the synchronized spiking of the individual neurons to high-level cognitive processes. Traditionally, the oscillatory nature of LFP dynamics motivates using Fourier methods, which

have dominated LFP research for the last several decades and currently constitute the only systematic framework for understanding the "brain waves." Yet, these methods are not well suited for handling the two fundamental attributes of biological signals: noise and nonstationarity, and may therefore obscure the actual physiological structure of the recorded data.
To address this problem, we developed an approach based on the Padé Approximation theory—a powerful novel technique that allows nuanced analyses of the LFP structure and opens a new perspective on the field. Specifically, our method possesses an impartial marker of the noise component, which allows identifying and removing the "noise shell" from the signal, uncovering its oscillatory part, and then investigating not only these individual components, but also studying the interplay between the noise and the oscillatory dynamics. In particular, our analyses reveal that the LFP oscillations recorded in rats consist of a small, discrete set of frequency-modulated waves, embedded into a weak noise background. We hypothesize that these structures, which we call *oscillons*, represent the "physical" brain waves, whereas "theta," "gamma" and other traditional waves are nothing but approximately resolved oscillons, bleared by the less powerful computational techniques. In the presentation, we discuss some basic properties of the hippocampal and the cortical oscillons recorded in wakefulness and in sleeping states.

Disclosures: Y.A.D. Dabaghian: None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.10/Y23

Topic: H.01. Animal Cognition and Behavior

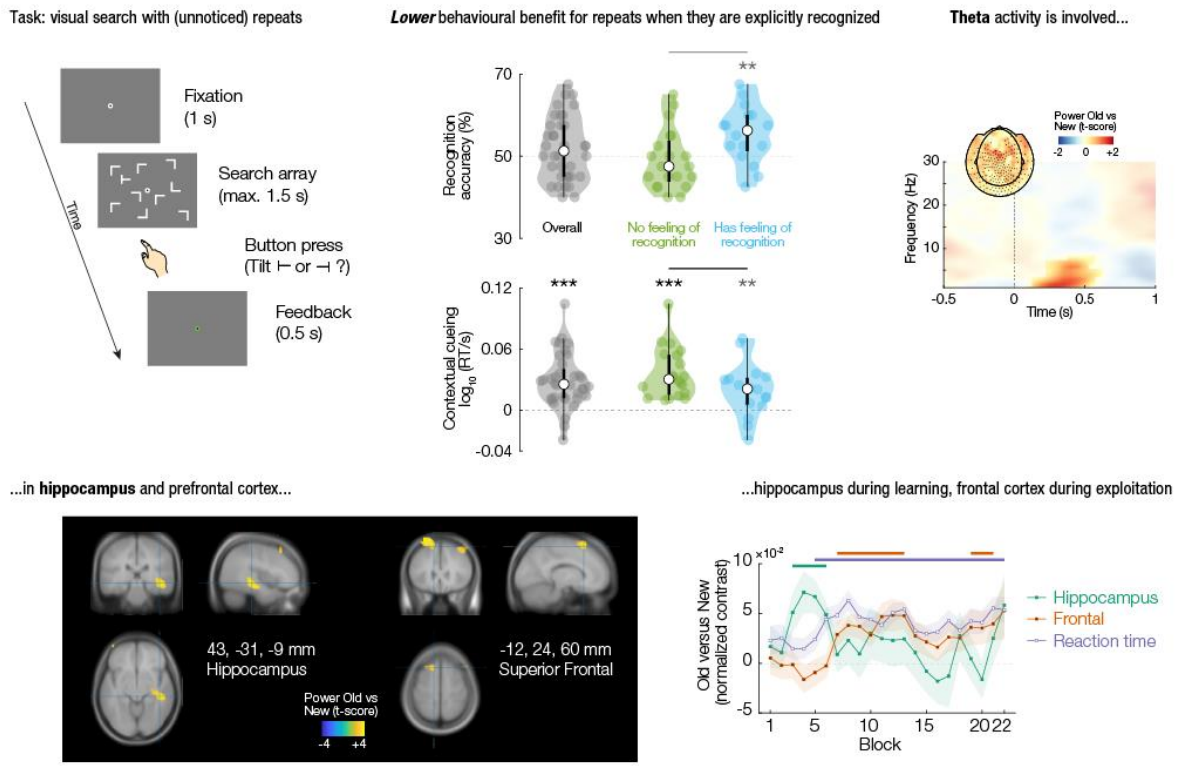
Support: NWO Veni Grant 016.Veni.198.065
NWO Vidi Grant 452-13-016
ERC Starting Grant 678286

Title: Implicit learning and exploitation of regularities involve hippocampal and prefrontal theta activity

Authors: *E. SPAAK, F. P. DE LANGE;
Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: Observers rapidly and seemingly automatically learn to predict where to expect relevant items when those items are repeatedly presented in the same spatial context. This form of statistical learning in visual search has been studied extensively using a paradigm known as contextual cueing. The neural mechanisms underlying the learning and exploiting of such

regularities remain unclear. We sought to elucidate these by examining behaviour and recording neural activity using magneto-encephalography (MEG) while observers were implicitly acquiring and exploiting statistical regularities. Computational modelling of behavioural data suggested that after repeated exposures to a spatial context, participants' behaviour was marked by an abrupt switch to an exploitation strategy of the learnt regularities. MEG recordings showed that the initial learning phase was associated with larger hippocampal theta band activity for repeated scenes, while the subsequent exploitation phase showed larger prefrontal theta band activity for these repeated scenes. Strikingly, the behavioural benefit of repeated exposures to certain scenes was inversely related to explicit awareness of such repeats, demonstrating the implicit nature of the expectations acquired. This elucidates how theta activity in the hippocampus and prefrontal cortex underpins the implicit learning and exploitation of spatial statistical regularities to optimize visual search behaviour.



Disclosures: **E. Spaak:** None. **F.P. de Lange:** None.

Poster

083. Cortical and Cortico-Hippocampal Circuits: Spatial Navigation I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 083.11/Y24

Topic: G.01. Appetitive and Aversive Learning

Title: Mapping spatial memory in fish

Authors: *C. CURRAN-ALFARO, W. M. SAIDEL;
Rutgers Univ., Camden, NJ

Abstract: Spatial memories are vital for a species survival. Short term memories are made and then consolidated into long term memories. The hippocampus is required in the mammalian brain for memory formation, fish do not have a cortical hippocampus. Despite this, fish are capable of spatial memory. Thus, there should be an analog to the mammalian hippocampus. However, currently, this analog has not been convincingly identified. Developmental differences of the neural tube between fish and mammals make comparisons by topography difficult. In an effort to locate the analog of the hippocampus, a learning task was conducted to force the formation of a memory to show regional changes in ATP demand in the brains of trained fish in comparison to naïve fish. An increase in ATP demand in the lateral ventral area of the dorsalis telencephali (Dlv) in the experimental group was noted, which was expected due to previous literature. However, an increased demand for ATP in the medial area of the dorsalis telencephali (Dm) amongst the experimental group appeared greater than observed in Dlv. This observation may suggest that Dm may be the primary focus not the Dlv in the mechanism involved with memory in fish.

Disclosures: C. Curran-Alfaro: None. **W.M. Saidel:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.01/Y25

Topic: H.01. Animal Cognition and Behavior

Support: JST PRESTO JPMJPR1882 (T.K.)
JSPS KAKENHI 19H04937, 17H05575, 17H05977, 17K14939 (T.K.), 18H05137, 17K19462, 16H04656 (K.M)
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the Nakajima Foundation (T.K.)
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Toray Science Foundation (K.M.)
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Title: Pathway-specific information outflow from the subiculum

Authors: *T. KITANISHI[1,2,4], R. UMABA[2,3], K. MIZUSEKI[1,2];
[1]Dept. of Physiol., [2]Ctr. for Brain Sci., [3]Dept. of Neurosurg., Osaka City Univ. Grad. Sch. of Med., Osaka, Japan; [4]Presto, JST, Kawaguchi, Japan

Abstract: The hippocampus processes multimodal information including place, speed, head direction, time, emotion, and memory. However, how such information is distributed to multiple downstream areas remains poorly understood. The subiculum is the major hippocampal output structure that receives hippocampal CA1 output and projects to multiple cortical/subcortical areas. Despite its anatomical importance, the nature of information distribution from the subiculum to downstream areas is largely unknown. We investigated this issue by optogenetically identifying the projection targets of individual subicular neurons during large-scale extracellular recordings in freely behaving rats. We first stereotaxically introduced channelrhodopsin-2-expressing viral vector and a 256-channel silicon probe into the dorsal subiculum, and also implanted up to four optical fibers targeting each of the subicular projection targets (i.e. nucleus accumbens, anteroventral thalamic nucleus, retrosplenial cortex, and medial mamillary body) in single rats. Then, two to three weeks after the surgery, while monitoring the firing activity of dozens of subicular neurons, we identified projection targets of the activity-monitored neurons by giving blue light pulses sequentially to each of the target areas. A proportion of subicular neurons reliably generated short-latency, low-jitter, antidromic spikes in response to the light irradiation, and were identified as projection neurons targeting the irradiated area. We then measured the information encoded by the projection neurons (e.g., place, head-direction, and working memory information), by monitoring neuronal activity during multiple behavioral tasks (open field, linear track, T-maze, and zigzag maze) and sleep. Preliminary analysis suggests that several types of information conveyed by subicular neurons are distributed in a target-specific manner during the tasks and sleep. Thus, our large-scale opto-electrophysiological approach allows us to uncover how multimodal information in the hippocampus is distributed to multiple downstream areas through the subiculum.

Disclosures: **T. Kitanishi:** None. **R. Umaba:** None. **K. Mizuseki:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.02/Y26

Topic: H.01. Animal Cognition and Behavior

Support: Norwegian Research Council - Project Establishment Support FRIPRO
NTNU internal funding

Title: The impact of NEIL3-dependent molecular mechanisms on neural coding of spatial memory

Authors: *N. KUNATH, P. BIGONAH, A. VANKOVA, M. BJØRÅS, J. YE;
Dept. of Clin. and Mol. Med., Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: Background. Spatial memory is encoded in the entorhinal-hippocampal spatial representation system, consisting of functionally distinct spatial cell types embedded in neural circuits. Understanding the underlying molecular, cellular and network mechanisms is a major goal in the field of neuroscience. NEIL3 belongs to the "Endonuclease VIII-like" ("NEIL") family of DNA glycosylases that initiates the Base Excision Repair (BER) pathway by recognizing and removing oxidative DNA base lesions. Emerging evidence suggests a role of NEIL3 beyond canonical BER in gene regulation via modulation of epigenetic DNA methylation. Interestingly, NEIL3 shows strong expression in neurogenic niches of the brain, especially during neurodevelopment. It also plays a crucial role in continuous adult and induced neurogenesis. In addition, mice lacking NEIL3 display a differential synaptic composition in the hippocampus and an impaired spatial learning phenotype in the Morris water maze. The impact of NEIL3-regulated molecular mechanisms on neural coding of spatial memory is yet to be characterized. **Objectives.** We aim to unravel the role of NEIL3 on place coding in the spatial representation system by (a) characterizing the postnatal maturation of NEIL3-depleted hippocampal neurons, (b) exploring the stability and plasticity of CA1 place cells in NEIL3-deficient mice and (c) identifying NEIL3-dependent molecular mechanisms underlying place cell function. **Methods.** We use immunohistochemistry and confocal microscopy to examine the postnatal maturation of hippocampal neurons in wildtype (wt) and NEIL3$^{-/-}$ mice. We record instant neuronal activity of CA1 place cells in freely moving wt and NEIL3$^{-/-}$ mice using a multiple single-unit extracellular recording method. We monitor stability and remapping of place fields in familiar and novel environments. We collect RNA and DNA samples from laser-dissected CA1 neurons of adult wt and NEIL3$^{-/-}$ mice for transcriptomic and epigenomic sequencing. **Results**. Postnatal maturation of hippocampal neurons is delayed in mice lacking NEIL3, mainly between postnatal day 8 and 14, which is a period crucial for hippocampal synaptogenesis. Maturation markers reach similar levels in wt and NEIL3$^{-/-}$ in adults. Place cells show regular firing fields and undergo normal global remapping in a novel environment in both NEIL3$^{-/-}$ and wt mice. However, place fields of NEIL3$^{-/-}$ place cells are not stable across different sessions in the familiar environment, corresponding to an impaired spatial memory phenotype. We are identifying CA1-specific gene transcription and DNA methylation profiles regulated by NEIL3 in adult animals.

Disclosures: N. Kunath: None. **P. Bigonah:** None. **A. Vankova:** None. **M. Bjørås:** None. **J. Ye:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.03/Y27

Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Behavior time-scale synaptic plasticity supports the manifestation of a conjunctive code in the hippocampus

Authors: *X. ZHAO[1], C.-L. HSU[1], N. P. SPRUSTON[2];
[1]Janelia Res. Campus, HHMI, Ashburn, VA; [2]HHMI Janelia Res. Campus, Ashburn, VA

Abstract: The brain can form distinct representations of the same environment based on the animal's memory and behavior. Pyramidal cells in hippocampal area CA1 have been shown to encode the position in T-mazes with firing rates modulated by the animal's past experience and/or future movement (i.e. 'splitter' cells, Wood et al. 2000 and Frank et al. 2000). However, how such a conjunctive code is formed in CA1 remains unclear. To address this question, we performed intracellular whole-cell recordings in mouse CA1 during virtual navigation in T-mazes. Mice were trained to run down a track containing task-specific cues (first part of the track), followed by a region lacking task-specific cues (second part), and a T junction (third part, requiring a right or left turn). In order to obtain a reward, mice had to turn into the correct arm, based on visual cues in the first part of the track. The visual scene in the second part of the track was strictly controlled to prevent distinct visual inputs determined by directional bias of the animal's locomotion. In this region, behavioral time-scale synaptic plasticity (BTSP) was induced by injecting current to elicit calcium plateau potentials. Our preliminary data show that BTSP induced after the mouse was cued to turn into one of the arms resulted in choice-specific, spatially tuned ramp depolarization in the vast majority of CA1 pyramidal cells tested. These results suggest that BTSP can support inheritance of a conjunctive code of position and choice-related information in CA1 from its presynaptic inputs.

Disclosures: X. Zhao: None. **C. Hsu:** None. **N.P. Spruston:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.04/Y28

Topic: H.01. Animal Cognition and Behavior

Support: HFSP grant RGY0088/2014
NSF CAREER IOS-1844935 to MvdM

Title: Shared representational geometry as an explanation for cross-subject prediction of place cell data from the rodent hippocampus

Authors: *H.-T. CHEN, J. R. MANNING, M. A. A. VAN DER MEER;
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Abstract: A fundamental challenge faced by any memory system is how related experiences should be organized: storing the details of individual experiences preserves potentially valuable details, but is storage-inefficient and hampers generalization, whereas treating all experiences as the same risks ignoring potentially important differences.
The rodent hippocampus is a model system for studying the neural basis of these computations. It can construct statistically independent representations across environments ("global remapping"; Alme et al. 2014) and assigns individual neuron firing fields to locations in an apparently random fashion (Rich et al. 2014). Similarly, "engram" studies suggest that the population of neurons allocated to a given experience is determined by a competition based on slowly fluctuating excitability levels among eligible neurons (Josselyn & Frankland, 2018). This relatively random mapping between hippocampal neurons and their coding properties implies that it should be challenging to predict hippocampal encoding of one experience in a given subject based on the neural encoding of experience in another subject. Contrary to this prediction, we find that by constructing a common representational space across rats ("hyperalignment"; Haxby et al. 2011), we can consistently predict data of "right" trials (R) on a T-maze in a target rat based on 1) the "left" trials (L) of the target rat, and 2) the relationship between L and R trials from a different source rat. These cross-subject predictions outperformed a number of control mappings, such as those based on permuted data that broke the relationship between L and R activity for individual neurons, and those based solely on within-subject prediction.
To better understand the basis for these successful cross-subject predictions, we simulated several scenarios incorporating different potential place cell properties: 1) cells only have a place field on either the L or R, 2) cells have independent probabilities of having fields on L and R, and 3) cells have independent probabilities of having L and R place fields, with the additional constraint that cells with both L and R fields must have fields in the same (relative) locations. Our simulations indicate that the results are not a simple consequence of either one or a mixture of these scenarios. This suggest the intriguing possibility of a representational geometry in the rodent hippocampus that is shared across subjects.

Disclosures: H. Chen: None. **J.R. Manning:** None. **M.A.A. van der Meer:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.05/Y29

Topic: H.01. Animal Cognition and Behavior

Support: Prince
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GIF

Title: Place cells demonstrate pattern completion deficits in hippocampal CA3 and pattern separation deficits in dentate-gyrus in Alzheimer's disease model mice performing a novel tactile-morph task

Authors: O. RECHNITZ, D. KHATIB, G. MORRIS, ***D. DERDIKMAN**;
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Abstract: Pattern completion and separation deficits have been previously used as measures for dementia in both humans and rodents. We wished to relate these phenomena to ensembles of place cells in mice, by following the dynamics of place cell remapping in a novel tactile-morph task, comparing Alzheimer's model (AD) to wild-type (WT) mice. We created a novel tactile-morph paradigm tailored to create a strong, non-geometric dependent, remapping effect. In this paradigm, mice were trained to run on an elevated linear track with interchangeable floors of fine or coarse texture, as we recorded place cell activity from the dentate gyrus (DG) or CA3. Training was complete when the population of recorded cells achieved clear distinction between fine and coarse conditions, while also presenting a stable representation of each condition. Following the training phase, mice were presented with a gradient of seven textures - fine to coarse, and vise-versa. The cell population showed a clear difference in representation dynamics between the WT and the AD model mice across the contextual-tactile gradient. We observed two key differences between groups: 1. A greater tendency of the DG of AD model mice to have a persistent time-dependent representation through the gradient, suggesting a pattern separation deficit; 2. In CA3, however, AD mice presented an opposing trend of increased remapping relative to WT mice, suggestive of a pattern completion deficit. We then wanted to test whether the driving force of pattern separation/completion deficits in the AD model were time or context dependent, and exposed the mice to a random-order change in texture. Both WT and AD model mice presented decreased representation stability between distant trials in either time, texture or a combination of the two. This, while maintaining a similar trend of an overall increased correlation in AD vs. WT in the DG and a decreased correlation in AD vs. WT in CA3. We conclude that AD mice show a representation deficit, which is related to both time and context - where the classical roles of both DG and CA3 in pattern separation and completion, respectively, are impaired.

Disclosures: O. Rechnitz: None. **D. Khatib:** None. **G. Morris:** None. **D. Derdikman:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.06/Y30

Topic: H.01. Animal Cognition and Behavior

Support: BBSRC grant: BB/P001726/1

Title: Cue control in head direction and place cells: Landmarks vs. barriers

Authors: A. E. SMITH[1], E. R. WOOD[1], ***P. A. DUDCHENKO**[2];
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Abstract: A basic property of both place and head direction cells is that their spatial firing is often anchored to salient visual landmarks within an environment. For example, in the traditional paradigm developed by Muller and Kubie (1987), displacement of a cue card on the wall of a cylindrical environment (in the absence of the animal), yields a corresponding shift in the firing location or direction of place and head direction cells, respectively, when the animal returns.
A second feature of the environment that may serve as a polarising cue for spatially tuned cells relates to its structure. This can be the shape of the environment, for example the corners and wall lengths of a rectangular enclosure. Alternatively, features within the environment, such as a large barrier, might provide a cue to distinguish locations and direction. Previous work has shown that place and boundary vector cells respond to the introduction of such barriers (Barry et al., 2006; Stewart et al., 2014), but it is unclear whether these exert directional stimulus control over the spatial firing of place cells and head direction cells.
To test this, we recorded from the medial entorhinal cortex/subiculum (n = 7) and hippocampus (n = 3) of male, Lister Hooded rats. Upon identification of a spatially tuned neuron, rats were tested in an octagonal environment either with a cue card affixed to one wall, or with a barrier in the center extending from one wall to three quarters of the distance to the opposite wall. In the cue card rotation sessions, rats were given a standard session, a session where the cue card was affixed to a wall 90 degrees from the standard session, and a return session, where the cue card was in the same place as the standard session. Between each of these sessions, the animal was removed from the octagon and placed in a holding bucket. The barrier rotation sessions were done in the same way: standard session - 90 degree shift of barrier session - return to standard session. The order in which these cue and barrier rotation sessions were conducted was varied across sessions, and for place cells the return sessions were omitted. Our preliminary results suggest that, at least for head direction cells, the cue card exerted stronger stimulus control over preferred firing directions compared to the barrier. Such a pattern of results, if substantiated across additional animals, may suggest that barriers are not equivalent to polarising landmarks in controlling the spatial firing of head direction and place cells.

Disclosures: **A.E. Smith:** None. **E.R. Wood:** None. **P.A. Dudchenko:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: H.01. Animal Cognition and Behavior

Support: Horizon 2020 - ERC - StG (638644)
Israel Science Foundation (2184/14)

Title: Long-term spatial memory is maintained in the presence of ongoing changes in hippocampal representations

Authors: N. SADEH, M. ZEMER, L. SHEINTUCH, L. BALILTI TORGMAN, A. RUBIN, *Y. ZIV;
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Abstract: The hippocampus is essential for spatial navigation and long-term episodic memory, but relatively little is known about the neural network mechanisms underlying memory's persistence over timescales that are longer than a few days. Hippocampal place cells have long been considered a neural substrate for long-term spatial memory. While the common view is that long-term spatial memory relies on a stable hippocampal spatial representation, recent work has revealed that the ensemble of CA1 place cells that encode a given familiar environment gradually changes over long time scales. In this work we asked: (1) To what extent are hippocampal spatial codes stable under conditions that require a stable long-term spatial memory? (2) To what extent does performance in a spatial memory task correlate with the stability of the hippocampal representation of the environment? (3) To what extent does performance in a spatial memory task correlate with the quality of the hippocampal representation of the environment? (4) Does re-learning to navigate in a familiar environment after weeks reinstate the same spatial representation that was present at the end of learning? To answer these questions, we used miniaturized microscopes to longitudinally track calcium dynamics of large populations of the same neurons in the hippocampal CA1 as mice (N=20) learned to navigate to a reward zone in a radial arm maze over five consecutive days, and again when they performed a memory test and allowed to relearn the environment 10 or 28 days later. We found that the hippocampal representation of the maze was dynamic despite the successful expression of long-term spatial memory, and that these changes in the spatial representation were more pronounced with elapsed time from the last day of learning. Interestingly, 28 days after learning, spatial memory performance was correlated with place code quality (spatial

information) but not with place code stability. Finally, re-learning to navigate the maze after 28 days was associated with a new spatial representation rather than a conversion back to the previously established representation. Taken together, our results suggest that spatial memory content is reliably preserved without relying on a stable hippocampal spatial representation.

Disclosures: **Y. Ziv:** None. **N. Sadeh:** None. **M. Zemer:** None. **L. Sheintuch:** None. **L. Balilti Torgman:** None. **A. Rubin:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

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Title: Global place encoding in a visually ambiguous environment

Authors: *H. Y. CHENG, D. W.-U. OVERINGTON, K. J. JEFFERY;
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Abstract: For efficient navigation, animals have to disambiguate visually similar local environments and locate themselves globally. To facilitate disambiguation, a neural substrate that conjunctively encodes both local and global environments may exist. A recent study suggests that the retrosplenial cortex (RSC) may be involved. Specifically, a subpopulation of cells in dysgranular RSC (dRSC), but not granular RSC (gRSC), showed directional-specific firing that 'flip' 180° between 180° visually rotated, differentially scented compartments. These bidirectional (BD) cells might reflect local visually-sensitive direction encoding, and are distinct from head direction (HD) cells which displayed single preferred firing direction (PFD) across compartments (global directional encoding; Jacob *et al*., 2016, Nat Neurosci, 20(2), 173-175). To understand how such differential direction information could arise, we examined the neuronal projections to dRSC and gRSC. dRSC receives significantly fewer projections from anterodorsal thalamus than gRSC, suggesting that BD cells in dRSC may receive less vestibular-weighted directional signal than gRSC. This might thus account for their lack of modulation by self-motion cues and increased sensitivity to the local visual scene. In addition, dorsal subiculum projects exclusively to gRSC, suggesting that gRSC may receive contextual information from hippocampus (via dorsal subiculum) that may help stabilizes PFD of HD cells in gRSC.
We next asked if the representation of place cells resemble that of BD or HD cells. As our

tracing data suggest a greater coupling between hippocampal output (via dorsal subiculum) and gRSC, we predicted that place cells would show representations similar to that in gRSC (HD cells; global encoding). Recording of place cells in the two-compartment context box revealed global encoding of the environment, with most place cells displaying either single or non-related fields across the two compartments. A small percentage of place cells displayed duplicated fields across compartments while almost no place cells displayed 180° rotated fields between the two compartments. Therefore, place cells displayed global encoding with only a small number of place cells showing sensitivity to the local environment.

Disclosures: H.Y. Cheng: None. **D.W. Overington:** None. **K.J. Jeffery:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.09/Y33

Topic: H.01. Animal Cognition and Behavior

Support: NSFC 31421003

Title: Oscillation activity of an item-location-retention task in the medial temporal lobe of non-human primates

Authors: *H. CHEN[1,2,3], Y. NAYA[1,2,4,5];
[1]Peking Univ., BEIJING, China; [2]Ctr. for Life Sciences, Peking Univ., Beijing, China; [3]Acadamy for Advanced Interdisciplinary Studies, Peking Univ., Beijing, China; [4]Sch. of Psychology and Cognitive Sciences, Peking Univ., Beijing, China; [5]IDG/McGovern Inst. for Brain Research, Peking Univ., Beijing, China

Abstract: The conventional insight regarding object-location integration processes in the medial temporal lobe (MTL) describes a convergence of ventral and dorsal pathways into the hippocampus (HPC) via the perirhinal cortex (PRC) and the parahippocampal cortex (PHC). However, recent visual neuroscience studies suggest that integration proceeds along the ventral pathway prior to reaching the HPC. In previous study, we presented that an object and its location information are combined in a stepwise manner from the ventral stream to MTL using an item-location-retention (ILR) task (*2018 SFN, Chen and Naya*). In the ILR task, we required the animals to encode a sample stimulus presented at one of the quadrants on a display for its identity and location information. Single-unit activities were recorded from the MTL areas as well as visual area TEv of two macaques while they performed the ILR task. We found populations of neurons exhibiting location signals as well as those exhibiting object signals in the TEv and its downstream MTL areas (i.e., PRC and HPC) when the animals foveated a particular object-place combination (i.e., foveal view condition). In all three areas, the two signals co-

existed in each area and also exhibited a convergence effect on responses of single neurons. We detected a significant integration effect between the object and fixation-triggered location signals in the TEv and PRC (i.e. Type I integration), and a significant integration effect between the object and sample-triggered location signals in PRC and HPC (i.e. Type II integration). In current analysis, we examined the local field potentials (LFPs) in the MTL areas and TEv. To quantify the location effect and its integration with the object identity, we subtracted the powers of LFPs in trials of a control task from those of the standard ILR task (i.e., foveal view condition). In the control task, a sample stimulus was presented randomly at one of four quadrants while the subject fixated at the center of display (i.e., peripheral view condition). We found a wide-spread beta-band like oscillation (10-30HZ) in PRC, PHC and HPC of the MTL as well as TEv during the sample presentation in the encoding phase of the standard ILR task. Further, when examined LFPs at the sites where Type II integration cells were recorded, we found a significant gamma-band like oscillation (70-90HZ) after the sample presentation in the encoding phase in HPC but not in PRC. These results may suggest different neural mechanisms between HPC and PRC even though they represent the same type of integration signal at single-cell level (i.e. Type II integration) when a subject encodes an object at a particular location on a background.

Disclosures: **H. Chen:** None. **Y. Naya:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

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Topic: H.01. Animal Cognition and Behavior

Support: ANR-17-0015-Navi-GPS
ANR-08-BLAN-0088-
ANR-11- LABEX-OO42

Title: Contrasting parietal and hippocampal activity during virtual navigation in the macaque

Authors: M. VERICEL[1], ***J.-R. DUHAMEL**[1], P. BARADUC[2], S. WIRTH[1];
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Abstract: Navigation relies on several brain areas. We compared single neuron activity recorded in the cortex in the intraparietal sulcus to neuronal activity in the hippocampus, in macaques performing a virtual navigation task. Animals searched the maze for a reward, based on its relative position with respect to landmarks. We carried out unsupervised clustering analysis on activity as a function of the animal's position. Activity patterns separated in a different manner in

the two regions. Parietal cortex cells formed stronger clusters of cells compared to the hippocampus and separated according to all possible actions in different parts of the maze: on paths, on turns, on path preceding reward, following reward. These clusters are consistent with previously described properties of cells (saccade, spatial attention, optic flow), and illustrate how response properties appear to parse out space in an active navigation task due to the recruitment of spatially selective actions during navigation. In the hippocampus, they were fewer meaningful cell clusters suggesting that patterns of activity were too diverse to be grouped in clusters. Our results show how two main structures involved in navigation complement each-other during active behaviour.

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Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

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Topic: H.01. Animal Cognition and Behavior

Support: NUS Memory Networks Program
Singapore Ministry of Education Tier 2 Research Fund
Singapore Ministry of Education Tier 3 Research Fund

Title: Place selectivity in the hippocampus of the non-human primate

Authors: ***H. TAN**[1], T. P. Y. NG[1], C. OWENS[1], C. LIBEDINSKY[2,1], S.-C. YEN[3,1]; [1]Singapore Inst. for Neurotechnology (SINAPSE), Singapore, Singapore; [2]Dept. of Psychology, Natl. Univ. of Singapore, Singapore, Singapore; [3]Dept. of Electrical and Computer Engineering, Natl. Univ. of Singapore, Singapore, Singapore

Abstract: The hippocampus is a key brain structure in the representation of navigational space. Place cells, hippocampal neurons that respond to allocentric location, are well-studied in the rodent model, and have also been identified in the hippocampus of multiple mammalian species, including humans. However, work in the hippocampus of non-human primates (NHPs) has revealed significant differences. Evidence of place selectivity in NHPs has been sparse, and in contrast with the strong phase dependence of rodent place cell activity on LFP theta oscillations, movement-related theta oscillations in NHPs, where reported, is intermittent (Leonard et al 2015). Separately, spatial view cells, neurons that respond allocentrically to the part of the environment being viewed, have been identified in NHPs. There is a need to reconcile these differences by concurrently analyzing place and spatial view selectivity in the same set of neurons, but the disambiguation of spatial view from place activity has been difficult in past work done in uni- or bi-directional linear tracks (Wirth et al 2019; Hazama Tamura 2019; Ono et

al 1991). The present study recorded hippocampal activity of a male macaca fascicularis with a 124-channel chronically implanted microelectrode array, while he performed a continuous, goal-directed navigation task within a virtual open maze with 6 fixed goal locations. Eye gaze was tracked concurrently with location in the maze. The animal completed 19 sessions of 400 correct trials each, and was able to navigate to randomly selected goal locations in 94.0 ± 1.17% (mean ± sem) of the trials in 9.22 ± 0.3 s, taking the shortest route in 82.5 ± 1.15% of those trials in 8.65 ± 0.3 s. 128 putative hippocampal cells were recorded, and place selectivity was found to be significant in 53.9% of the recorded cells, independent of path density within the maze, by comparing the spatial information content in the data to 10,000 circularly shifted shuffled spike trains. Additionally, the cell responses were analyzed with respect to heading direction and spatial view. Local field potentials were also analyzed for the presence of theta and gamma frequencies.

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Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

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Support: NIMH R01-MH117763
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NIDA R21DA041791
DARPA W911NF-14-2-0043

Title: A value map in primate hippocampus during reward-based learning

Authors: *E. B. KNUDSEN, J. D. WALLIS;
Univ. of California Berkeley, Berkeley, CA

Abstract: Despite a long-standing idea that neurons in hippocampus (HPC) encode cognitive maps, the evidence for this assertion is rather weak, relying mainly on the presence of neuronal responses that discriminate non-spatial information rather than the representation of a map per se. We previously showed that HPC causally interacts with orbitofrontal cortex (OFC) to enable learning in a task that required subjects to track reward-predictive cues as they gradually changed in value (reward probability) in order to guide decision making. While OFC neurons encoded the values of the cues themselves, we hypothesized that the role of HPC might involve mapping the relationship between values as they changed, which would enable subjects to exploit task structure to optimize learning, rather than relying on trial-and-error learning. To test this idea, we

designed several structured paths through a three-cue value space while we recorded large (~80-100) ensembles of HPC neurons in two monkeys.
In the simplest path (circular), approximately 25% of HPC neurons exhibited value place-preference. These value place cells had similar properties to those recorded during spatial navigation, such as consistent firing in one location of the value space across multiple path traversals. When new reward-predictive cues were introduced, we observed that place fields remapped to new regions of the value space. When returning to the original pictures, there was a reemergence of the original place fields. In more complex spaces, place representations extended into the third dimension and many fields were contingent upon the direction of approach along the value trajectory. Together, these results provide the first direct evidence that HPC contains 'value place neurons', whereby HPC uses a high-dimensional space to encode the value of stimuli relative to one another, consistent with a map of a cognitive parameter.

Disclosures: E.B. Knudsen: None. **J.D. Wallis:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.13/Y37

Topic: H.01. Animal Cognition and Behavior

Support: NSF DGE 1752814

Title: Manipulating interactions between model-based and model-free reinforcement learning systems in nonhuman primates

Authors: *C. F. FORD[1], J. D. WALLIS[2];
[1]Helen Wills Neurosci. Inst., [2]Dept. of Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: Reward-based learning involves at least two distinct learning systems: model-free (MF) and model-based (MB) reinforcement learning (RL). MF-RL is computationally efficient at the expense of flexibility, while MB-RL draws upon executive resources and sacrifices efficiency for flexibility. Although experimental evidence has demonstrated a role for both strategies in learning and decision-making, how the brain arbitrates between the two systems remains unknown. Recent work in humans has shown that experimentally controllable factors like uncertainty, stress, and cognitive demand can push behavior towards one RL strategy or another. However, noninvasive neuroimaging methods used in human subjects lack the spatiotemporal precision required to fully understand the underlying brain mechanisms. To address this limitation, we have adapted a paradigm from the human literature for use in non-human primates that can manipulate how strongly subjects rely on MF or MB RL. Two male monkeys (*Macaca mulatta,* 5.5 years old, 7.8 and 8.2 kg) learned the values of two images via a

two-step decision-making task, while also engaging in a spatial WM task. By independently varying the difficulty of the WM task (i.e., cognitive load) in this dual-task setting, we could influence subjects' relative reliance on MF- or MB- RL on a session-by-session basis. We fit RL models to choice behavior to estimate the weight of MB (relative to MF) values given the current task demands. Much like humans, the monkeys relied more heavily on MF RL when cognitive load was high. In future work, we will use this paradigm while simultaneously recording from multiple brain areas implicated in MB and MF RL to study how neuronal dynamics drive RL strategy selection. Orbitofrontal cortex (OFC) exists at the intersection of limbic and corticostriatal pathways associated with MB and MF RL, and so we will examine how OFC interacts with other brain regions as a function of RL strategy weight.

Disclosures: C.F. Ford: None. **J.D. Wallis:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

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Support: NIMH R01-MH097990

Title: OFC representations influence choice accumulation in ACC

Authors: *Z. Z. BALEWSKI, J. D. WALLIS;
Univ. of California Berkeley, Berkeley, CA

Abstract: One challenge in studying value-based decision-making is that, although the output of the process is measurable (a choice), the process itself is unobservable. Prior work in our lab (Rich and Wallis, Nat Neuro 2016) attempted to measure the underlying decision-making process by decoding it with single trial resolution from neural activity. We used a linear discriminant analysis to decode from monkey OFC which of four values associated with four reward-predictive cues was currently being represented in neural activity. When choosing between two of these cues, the decoder flip-flopped between the available options, consistent with the animal evaluating each option in turn. However, there was no evidence that the flip-flops were directly linked to the choice response.
The goal of this project is to ask how the vacillations in OFC are translated into decisions. The ACC is a good candidate region for this computation: it is strongly anatomically connected to OFC, and it shares many response properties with OFC, but additionally encodes motor information. We simultaneously recorded large ensembles of OFC and ACC units (average 50 units) using multi-site linear probes. We found discrete flip-flopping between value states in OFC replicating our original study. We also observed a large proportion of ACC neurons whose

firing rates gradually ramped to the chosen option. Finally, we found that the states decoded from OFC explained additional variance in the ramping of ACC neurons.
Our results are consistent with an evidence accumulation model of value-based decision-making. In this model, OFC is responsible for sequentially representing the value of available options, which in turn influences the rate of accumulation of evidence in ACC for choosing either alternative.

Disclosures: Z.Z. Balewski: None. **J.D. Wallis:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 1R01NS104917

Title: Strengthened temporal coordination within pre-existing sequential cell assemblies supports trajectory replay

Authors: *U. FAROOQ, J. SIBILLE, K. LIU, G. DRAGOI;
Psychiatry and Neurosci., Yale Univ., New Haven, CT

Abstract: A central goal in learning and memory research is to reveal the neural substrates underlying episodic memory formation. The hallmark of sequential spatial trajectory learning, a model of episodic memory, has remained equivocal, with proposals ranging from de novo creation of compressed sequential reactivations/replay from blank-slate networks to selection of pre-existing compressed preplay sequences. Here we show that increased millisecond-timescale activation of cell-assemblies expressed during de-novo sequential experience and their increased firing-rate correlations can explain the difference between post-experience trajectory replay and robust preplay. This increased activation results from an improved neuronal tuning to specific cell-assemblies, higher recruitment of experience-tuned neurons into pre-existing cell-assemblies, and increased recruitment of cell-assemblies in replay. In contrast, changes in overall neuronal/cell-assembly temporal order within extended sequences do not account for sequential trajectory learning. We propose the coordinated strengthening of cell-assemblies played sequentially on strong pre-existing temporal frameworks could support rapid formation of episodic-like memory.

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Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

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Program #/Poster #: 084.16/Y40

Topic: H.01. Animal Cognition and Behavior

Support: Whitehall Foundation
Brain and Behavior Foundation NARSAD
Charles H. Hood Foundation

Title: Preconfigured patterns are the primary driver of offline multi-neuronal sequence replay

Authors: *K. LIU, J. SIBILLE, G. DRAGOI;
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Abstract: Spontaneous neuronal ensemble activity in the hippocampus is believed to result from a combination of preconfigured internally-generated dynamics and the unique patterns of activity driven by recent experience. Previous research has established that preconfigured sequential neuronal patterns (i.e., preplay) contribute to the expression of future place cell sequences, which in turn contribute to the sequential neuronal patterns expressed post-exploration (i.e., replay). The relative contribution of preconfigured and of experience-related factors to replay and to overall sequential activity during post-run sleep is believed to be highly biased toward the recent run experience, despite never being tested directly. Here, we use multi-neuronal sequence analysis unbiased by firing rate to compute and directly compare the contributions of internally-generated and of recent experience-driven factors to the sequential neuronal activity in post-run sleep in naïve adult rats. We find that multi-neuronal sequences during post-run sleep are dominantly contributed by the pre-run preconfigured patterns and to a much smaller extent by the place cell sequences and associated awake rest multi-neuronal sequences experienced during de novo run session, which are weakly and similarly correlated with pre- and post-run sleep multi-neuronal sequences. These findings indicate a robust default internal organization of the hippocampal network into sequential neuronal ensembles that withstands a de novo spatial experience and suggest that integration of novel information during de novo experience leading to lasting changes in sequential network patterns is much more subtle than previously assumed.

Disclosures: K. Liu: None. **J. Sibille:** None. **G. Dragoi:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.17/Y41

Topic: H.01. Animal Cognition and Behavior

Support: ISF Grant FIRST Program
The Rapaport Research Institute Grant

Title: Head direction representation in the hippocampal formation of Japanese quails

Authors: *K. KRIVORUCHKO[1], E. BEN-YISHAY[1], N. ULANOVSKY[2], D. DERDIKMAN[1], Y. GUTFREUND[1];
[1]The Technion, Haifa, Israel; [2]Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Since the seminal discovery of hippocampal place cells in the 1970s' various types of cells were discovered in the hippocampus of rodents and its related structures (hippocampal formation) such as head-direction cells, grid cells and speed cells. However, one important question remained unresolved: To what extent similar cells can be found in other, particularly, non-mammalian species? Here we investigated the existence of such cells in birds. Specifically, we used adult Japanese quails (Coturnix Japonica). The quail is a useful model animal as they are ground dwelling foraging birds, which are easy to handle and raise. The dorsal pallium of birds is considered, based on anatomical, developmental and molecular grounds to be the avian hippocampal formation (HPF). We implanted custom-designed 4-tetrodes microdrives above the left HPF of quails. The quails were allowed to forage in a square 1X1 meter arena while electrophysiological data was collected with a Neuralynx system (tethered) or with a neurologger recording system (wireless). The 2D head direction and position in the arena were sampled at a rate of 60 points/sec by tracking two colored LEDs attached to the quail's head. We recorded about 1400 putative single units in 192 recording sessions of 10-20 minutes in the left HPF of 16 quails. About 10% of the recorded single units showed statistically significant ($p<0.01$) modulation of firing rate by the head pointing direction in allocentric coordinates. To check for temporal and spatial stability of the head-direction tuning, we analyzed separately the first and second halves of the session, the east and west halves of the arena and the center and near-borders of the arena. In the majority of cells, the head direction preference was maintained stable across the recording session and across different regions of the arena. Moreover, some cells maintained stable tuning over different recording sessions in the same day. In addition, we analyzed the speed of the quail and found that in most cells speed had no influence on the head-direction tuning curve. Our findings support the existence of an allocentric head-direction representation in the quail hippocampus. To our knowledge this is the first demonstration of head-direction cells in an avian species.

Disclosures: **K. Krivoruchko:** None. **E. Ben-Yishay:** None. **N. Ulanovsky:** None. **D. Derdikman:** None. **Y. Gutfreund:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.18/Y42

Topic: H.01. Animal Cognition and Behavior

Support: ISF grant BIKURA program
The Rappaport Research Institute Joint Grant

Title: Space and flight-direction representation in the dorsal pallium of barn owls

Authors: A. AGARWAL[1], A. SAREL[2], N. ULANOVSKY[2], D. DERDIKMAN[1], *Y. GUTFREUND[1];
[1]Technion - Israel Inst. of Technol., Haifa, Israel; [2]Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Studies of spatial representation in the hippocampus focus on mammalian species, mostly rodents. Birds diverged from the mammalian lineage ~300 million years ago - yet they demonstrate remarkable cognitive capabilities, including excellent spatial memory and navigation. Elucidating the role of the avian hippocampus will contribute to our understanding of the evolution of animal cognition in general, and of hippocampal function in particular. The hippocampal formation of mammalian species contains spatial cells such as place cells, grid cells, and head-direction cells. In contrast, to date, we know very little about how avian hippocampal neurons may contribute to the avian cognitive map of space. Barn owls are nocturnal birds whose visual and auditory systems have been extensively studied and are large enough to carry a wireless recording apparatus in flight. The dorsal pallium of birds is considered as the avian hippocampal formation (HPF), based on anatomical, developmental and molecular grounds. We implanted 4-tetrodes microdrives in the dorsal pallium of three barn owls. Single units were recorded and isolated using a 16 channel wireless neural-logger. The owls were trained to fly back and forth between two perches in a 7 x 5 x 3 meter room. The owl's 3D position was extracted from video tracking of infrared reflectors rigidly attached to the owls head. We recorded more than 200 single neurons in the frontal portion of the left HPF. A substantial number of neurons showed strong and significant modulations of firing rate by the spatial behavior of the owl. Three types of firing patterns were identified: 1) Flight-direction cells, firing considerably stronger during one direction of flight. 2) Place/direction cells, firing considerably stronger when the owl crossed a certain position during flight to one direction but not the other. 3) Perch cells, firing considerably stronger when the owl was standing on one of the two perches. In most neurons the preferred direction and/or place were stable during the 20 minutes recording sessions and were also stable to changes in ambient light conditions and to

changes in the position of a salient object in the room. Our findings support the existence of place representation in the barn owl's brain, akin to rodents. To our knowledge this is the first demonstration of place coding in a flying avian species.

Disclosures: **A. Agarwal:** None. **A. Sarel:** None. **N. Ulanovsky:** None. **D. Derdikman:** None. **Y. Gutfreund:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.19/Y43

Topic: H.01. Animal Cognition and Behavior

Support: R21 AA024983
P50AA022534
5 T32AA014127-15

Title: Hippocampal CA1, CA3, and dentate gyrus place cell firing characteristics in a rat model of moderate prenatal alcohol exposure

Authors: *R. E. HARVEY[1], L. E. BERKOWITZ[2], D. D. SAVAGE II[4], D. A. HAMILTON[5], B. J. CLARK[3];
[1]Psychology, Univ. of New Mexico, albuquerque, NM; [2]Psychology, [3]Psychology Dept., Univ. of New Mexico, Albuquerque, NM; [4]Neurosciences, Univ. of New Mexico Sch. of Med., Albuquerque, NM; [5]Univ. New Mexico, Albuquerque, NM

Abstract: Spatial memory and navigation impairments are common following prenatal alcohol exposure (PAE) in humans and in animal models. The hippocampus, which displays synaptic alterations following moderate doses of PAE (60-80mg/dl), contains neuron populations in the CA1, CA3, and dentate gyrus (DG) subfields that are highly modulated by environmental locations called place cells. Because hippocampal neuronal signals are critical for accurate spatial navigation, we investigate whether the firing characteristics of hippocampal place cells are altered following moderate PAE. To test this hypothesis, we performed electrophysiological recordings from the hippocampus (CA1, CA3, and DG) of adult male rats exposed to either moderate amounts of ethanol or saccharin throughout gestation. Hippocampal neural activity was monitored in two behavioral paradigms in which rats performed laps to each end of a narrow linear track (120 x 9cm) or while randomly foraging in a circular open field (76cm in dia). Similar numbers of hippocampal place cells were identified in both PAE and control rats, and further analysis revealed that CA1 place cells from PAE rats did not differ from control place cells on a wide variety of firing characteristics in both linear track and open field. In contrast, place fields from CA3/DG place cells following PAE were substantially degraded compared to

control place fields in both environments. Specifically, spatial information content, sparsity, field width, and peak firing rate were significantly different in PAE groups compared to control animals. We also investigated the impact of moderate PAE on hippocampal population oscillatory activity in theta and gamma frequency ranges. Our preliminary results suggest that phase amplitude coupling between theta and slow-gamma frequencies was diminished in CA1 of PAE rats. Taken together, our preliminary findings suggest that moderate PAE may produce selective deficits in place cell signaling in CA3/DG cell populations and oscillatory activity in CA1 of the hippocampus. The results will be discussed in relation to the hypothesis that deficits in hippocampal neural signaling underlie the consistent spatial behavior impairments observed after PAE.

Disclosures: R.E. Harvey: None. **L.E. Berkowitz:** None. **D.D. Savage II:** None. **D.A. Hamilton:** None. **B.J. Clark:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.20/Y44

Topic: H.01. Animal Cognition and Behavior

Title: Disruption of the anterior thalamic head direction cell network impairs the hippocampal place signal

Authors: R. E. HARVEY[1], L. E. BERKOWITZ[2], ***B. J. CLARK**[3];
[1]Dept. of Psychology, [2]Psychology, [3]Univ. of New Mexico, Albuquerque, NM

Abstract: Navigation depends on the interaction of multiple neural systems that are thought to encode an animal's direction and location in space. Two of which include head direction (HD) cells which fire maximally at a single head orientation and place cells which fire maximally at single locations throughout the environment. To better understand how directional information is used in the formation and function of place cells, we sought to determine whether place fields of hippocampal CA1-CA3 place cells would be altered following NMDA lesions or muscimol inactivation of the anterior thalamic nuclei (ATN). Rats were trained to perform laps on a circular track (360 cm in length) or randomly forage in a large open square arena (100 x 100 cm). Preliminary results show that the formation of CA1-CA3 place fields is drastically diminished following NMDA lesions of the ATN, and the place fields that did emerge contained substantially less spatial information on the circular track and in the open arena. Interestingly, fields from CA3 uniquely displayed temporal instability and were only active on a subset of laps on the circular track. Further, muscimol inactivation of the ATN, which temporally disrupts activity, altered CA1 place fields on the circular track and in the open arena. Specifically, several place cells had fields that fragmented, remapped, widened, changed in rate, or were silenced

during ATN inactivation. Interestingly, both lesion and inactivation of the ATN spared hippocampal theta rhythmicity. Together, ATN manipulation significantly disrupted hippocampal place cell characteristics indicating that the HD signal via the ATN is necessary for the generation and function of place cell activity.

Disclosures: R.E. Harvey: None. **L.E. Berkowitz:** None. **B.J. Clark:** None.

Poster

084. Hippocampal and Cortical Circuits: Memory, Head Direction, and Spatial Codes

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 084.21/Z1

Topic: H.01. Animal Cognition and Behavior

Support: AARG-17-531572

Title: Characterization of cortical and thalamic head direction cells in the TgF344-AD rat model of Alzheimer's disease

Authors: *L. E. BERKOWITZ[1], R. E. HARVEY[2], M. GABALDON-PARISH[1], V. J. ROY[1], B. J. CLARK[1];
[2]Psychology, [1]Univ. of New Mexico, Albuquerque, NM

Abstract: Spatial navigation and memory are impaired in early stages of Alzheimer’s disease (AD), and may be a defining behavioral marker of preclinical AD. The TgF344-AD rat model of AD has been shown to exhibit similar behavioral and pathological features to humans and thus serves as an excellent model to investigate mechanisms of spatial navigation impairment in AD. Previously, we found that 10 month old TgF344-AD rats make progressively less spatially precise movements during a reference memory variant of the Morris water maze. In addition, TgF344-AD rats also exhibit progressive pathology in cortical areas involved in the coding of head direction. Thus, this study sought to characterize directionally modulated cell types in the posterior cortex (retrosplenial, postsubiculum, and visual association area 2) and the anterior thalamus in 10 - 13 month female TgF344-AD rats and aged matched F344 controls. Rats performed a pellet chasing task within a cylinder fitted with a white cue card during recordings. In some sessions, the cue card was rotated to assess landmark control. Unimodal and multimodal directionally modulated cells were identified in both groups. Preliminary evidence indicates that directionally modulated cells located in the posterior cortex of TgF344-AD rats tend to fire in bursts, are more directionally stable, and exhibit more finely tuned directional firing characteristics relative to cells recorded from controls. Importantly, these cells also demonstrated intact response to cue manipulations whereby directional tuning was anchored relative to the position of the cue card. Directionally modulated cells recorded in the anterior thalamus of TgF344-AD rats also exhibit more finely tuned directional firing, while basic firing

characteristics, directional stability, and response to cue rotation were like that of control rats. These results will be discussed in relation to the hypothesis that head direction cell dysfunction underlies spatial impairments in Alzheimer's disease.

Disclosures: L.E. Berkowitz: None. **R.E. Harvey:** None. **M. Gabaldon-Parish:** None. **V.J. Roy:** None. **B.J. Clark:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.01/Z2

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH115604-03
NIH Grant R01DA044761-03

Title: Disynaptic, VTA-mediated, cerebellar modulation of the prefrontal cortex

Authors: *J. VERA[1], M. OÑATE[1], C. CHEN[2], V. LOVALLO[1], K. KHODAKHAH[1];
[1]Dept Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Harvard Med. Sch., Boston, MA

Abstract: The cerebellum (Cb) has been associated with cognitive disorders that potentially affect the medial prefrontal cortex (mPFC), such as schizophrenia and autism. However, how the cerebellum affects the mPFC remains to be established. The mPFC is thought to be involved in decision-making processes that guide behavior based on predicted outcomes. The ventral tegmental area (VTA) is a key region of the brain reward system that provides reward-related signals to the mPFC via dopaminergic and glutamatergic projections. We have recently shown that the Cb sends direct excitatory projections to the VTA, raising the possibility that there might be a disynaptic pathway from the Cb to the mPFC via the VTA. Here we describe experiments aimed at delineating the anatomical and functional properties of the Cb->VTA->mPFC circuit in the mouse brain. Using intersectional viral double tagging, we found that a number of mPFC-projecting VTA neurons also received direct synaptic inputs from the Cb, thereby anatomically confirming the presence of this disynaptic circuit. A large fraction, but not all, of these neurons within the VTA were dopaminergic (TH+), suggesting that a fraction of the projections might be glutamatergic. Using *in vivo* recording in head-fixed mice, we found that optogenetic stimulation of Cb axons in the VTA effectively drove the activity of half of mPFC cells recorded, with an average latency of 28 ± 10 ms (mean ± SD). In agreement with the anatomical data, the evoked response was composed of a transient fast (10-20 ms) increase in the firing rate of the recorded neurons that was often followed by a sustained (100-400 ms) increase, or reduction in firing, consistent with the involvement of both glutamatergic and dopaminergic VTA projections. Moreover, fiber photometry experiments showed that optogenetic stimulation of Cb axons in the

VTA evoked calcium transients in this region as well as fluctuations of dopamine levels in the mPFC, supporting the functional connectivity of the proposed circuit. Collectively, our results show the presence of an effective, VTA-mediated, disynaptic projection from the cerebellum to the PFC. This pathway may contribute to cognitive processing, and might provide a link between cerebellar dysfunction and mental disorders.

Disclosures: J. Vera: None. **M. Oñate:** None. **C. Chen:** None. **V. Lovallo:** None. **K. Khodakhah:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.02/Z3

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01DA044761-02
NIH Grant R01MH115604-02

Title: Mapping cerebellar connectivity with the prefrontal cortex via the ventral tegmental area

Authors: *M. OÑATE, V. LOVALLO, N. WAZEED, K. KHODAKHAH;
Dept Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Increasing evidence show that the cerebellum (Cb), in addition to its role in planning and execution of movement, also contributes to cognitive functions. Cerebellar dysfunction has been implicated in mental disorders that involve impairments in the reward system, including autism spectrum disorder (ASD), addiction and schizophrenia, but the causal link remains to be established. Recently, we showed that the cerebellum sends monosynaptic excitatory projections to the ventral tegmental area (VTA), one of the major brain regions that processes and encodes reward-related information. Behavioral experiments in mice suggest that cerebellar inputs to the VTA are rewarding and required for a social behavior task. The VTA sends direct dopaminergic and non-dopaminergic projections to the medial prefrontal cortex (mPFC), a brain area involved in decision-making and behavioral flexibility. The mPFC has also been involved in pathologies such as ASD and schizophrenia, suggesting a possible Cb → VTA → mPFC disynaptic circuit. To complement our *in vivo* electrophysiological recordings results that support the presence of disynaptic cerebellar projections to the mPFC via VTA, here we describe efforts aimed at anatomically delineating this pathway. We studied the projections of Cb-targeted VTA cells to the mPFC using an intersectional transynaptic viral tracing. We injected AAV.Cre virus in the deep cerebellar nuclei (DCN) of *RCE* mice (harboring a R26R CAG-boosted EGFP reporter allele with a *loxP*-flanked STOP cassette upstream of the EGFP gene), to identify all the postsynaptic targets of the cerebellum through EGFP expression. Concurrently, we injected a

retro Cre-dependent virus expressing tdTomato in the mPFC, to target all neurons that project to the mPFC, and receive inputs from DCN (that received AAV.Cre transynaptically, expressing EGFP and tdTomato). This approach allows for identification of disynaptic cerebellar inputs to the mPFC. We found double positive neurons both in the VTA, and also in the thalamus, demonstrating the presence of at least two parallel disynaptic pathway connecting the DCN with the mPFC. We performed retrograde tracing using G-deleted rabies virus in DAT-Cre and GAD-Cre mice to identify the cellular identity of Cb-targeted VTA neurons, and to establish the spatial organization of DCN-projecting neurons. We found that neurons from different regions of the DCN projected to dopaminergic and GABAergic neurons in the VTA. Together, these results support the presence of disynaptic cerebellar projections to mPFC via the VTA. Dysfunction of this pathway might contribute to cognitive and mental disorders in which the cerebellum is implicated.

Disclosures: **M. Oñate:** None. **V. Lovallo:** None. **N. Wazeed:** None. **K. Khodakhah:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.03/Z4

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01DA044761-02
NIH Grant R01MH115604-01A1

Title: Neuroanatomical characterization of cerebellar monosynaptic projections to the substantia nigra pars compacta and pars reticulata

Authors: M. OÑATE, J. VERA, S. WASHBURN, ***V. LOVALLO**, N. WAZEED, K. KHODAKHAH;
Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Recent research has shown direct and reciprocal connections between the cerebellum and basal ganglia, suggesting that these two circuits work in coordination to organize movement. Functional data from our laboratory show that the cerebellum sends monosynaptic excitatory projections to both substantia nigra *pars compacta* (SNc), and *pars reticulata* (SNr), targeting dopaminergic and non-dopaminergic cells and driving their activity on a fast time scale, suggesting that cerebellar inputs might contribute to dopamine fluctuations in the striatum. However, a number of questions remain unresolved, namely: 1) which cerebellar nuclei give rise to these projections, 2) whether the same cerebellar nuclei project to both SNc and SNr, and 3) what is the spatial distribution of targeted cells. Using a combination of anterograde and retrograde transynaptic viral tracing in the mice, we have sought to address these questions. We

injected *RCE:loxP* mice (harboring the R26R CAG-boosted EGFP (RCE) reporter allele with a *loxP*-flanked STOP cassette upstream of EGFP) with AAV.Cre targeting the entire deep cerebellar nucleus (DCN) to trace monosynaptic cerebellar projections. We found DCN-targeted neurons in the SNc and SNr, a high proportion of which were TH-positive (presumed dopaminergic) in the SNc, and conversely non-dopaminergic (TH-negative) in the SNr. To study where in the DCN these projections originated from, we performed rAAV retro Cre injections in *RCE:loxP* mice in either the SNc or the SNr. With this approach, we found that all three cerebellar nuclei (lateral, interposed and medial) contribute to these projections. To test the functional connectivity of the proposed circuit, we used fiber photometry recordings in mice walking in a circular treadmill, finding that optogenetic activation of DCN axons increase the activity of SN neurons and evoke dopamine fluctuations in the striatum. These results confirm the presence of direct monosynaptic projections from the cerebellum to the SNc and SNr, and suggest that the cerebellum can rapidly and effectively modulate these two midbrain regions, thereby influencing dopamine levels within the basal ganglia. Further delineation of the neuroarchitecture of these circuits would contribute to our understanding of mechanisms responsible for generating voluntary movements and motivate behaviors under physiological conditions, and also might help us understand the role of this pathway in disease.

Disclosures: **M. Oñate:** None. **J. Vera:** None. **S. Washburn:** None. **V. Lovallo:** None. **N. Wazeed:** None. **K. Khodakhah:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.04/Z5

Topic: H.01. Animal Cognition and Behavior

Support: R21MH116330

Title: Probing individual differences to dissect lateral orbitofrontal cortex contributions to distinct perseverative behaviors

Authors: ***E. E. MANNING**[1], X. LI[1,2], S. E. AHMARI[1];
[1]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [2]Tsinghua Univ., Beijing, China

Abstract: Neuroimaging studies implicate orbitofrontal cortex (OFC) dysfunction in obsessive compulsive disorder (OCD), with OFC hyperactivity observed during symptom provocation, and impaired OFC recruitment observed during tasks probing perseverative decision-making including reversal learning. *In vivo* imaging in the Sapap3 knockout mouse (KO), which displays OCD-relevant perseverative grooming and perseverative incorrect responding on a reversal learning task, can be used to determine whether overlapping or distinct activity patterns in OFC

contribute to these behaviors. Male and female mice were injected with virus encoding fluorescent calcium indicator (AAV5-hysn-GCaMP6f) and implanted with gradient-index (GRIN) lenses in lateral OFC (lOFC) to visualize neural activity using Inscopix miniature microscopes (n=12KO/8 wildtype (WT) littermate controls, ~5 months of age). Calcium imaging was performed during grooming assessment and reversal learning, and aligned to behaviors of interest (correct/incorrect responses, initiation/termination of grooming). Time spent engaging in compulsive grooming varied across individual KO mice (7-70% of time spent grooming), and increased severity of this phenotype was associated with more interrupted grooming patterns (increased grooming state transitions per grooming bout). In Sapap3-KO mice, more interrupted grooming patterns were associated with a larger population of lOFC neurons being activated at the start and end of grooming bouts (R^2=0.64, p<0.01). During reversal learning a subset of KOs (n=6) showed elevated perseverative incorrect responding; no correlation was seen between this behavior and levels of perseverative grooming. In KOs, elevated incorrect responding was correlated with a larger proportion of neurons being suppressed during incorrect responses (R^2=0.62, p=0.007). These data suggest that Sapap3-KOs show distinct patterns of lOFC activity change associated with severity of perseverative grooming versus reversal learning. Ongoing experiments are determining whether distinct striatal output populations contribute to these different behaviors.

Disclosures: E.E. Manning: None. **S.E. Ahmari:** None. **X. Li:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.05/Z6

Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01MH104255-05

Title: Assessing the role of direct and indirect pathway projecting spiny projection neurons in compulsive behavior

Authors: *S. C. PIANTADOSI, V. L. CORBIT, J. R. HYDE, B. CHAMBERLAIN, S. E. AHMARI;
Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Compulsive behaviors are hallmark symptoms of Obsessive Compulsive Disorder (OCD), and are often present in other severe neuropsychiatric illnesses. Aberrant activity in cortico-striatal circuitry has been linked to compulsive behavior in both correlative studies in humans and causal studies in rodents. Using head-mounted miniature microscopes for *in vivo* calcium imaging (Inscopix), we sought to determine the role of direct pathway projecting

dopamine D1-positive spiny projection neurons (D1-SPNs) and indirect pathway projecting dopamine D2-receptor positive SPNs (D2-SPNs) in mediating compulsive behavior in mice with a highly penetrant compulsive grooming phenotype (*Sapap3*-KO mice). Further, we evaluated how the first-line pharmacotherapy fluoxetine altered activity of these genetically distinct cell populations.
To visualize calcium activity in the combined population of D1-and D2-SPNs, *Sapap3* knockout (KO) mice were injected with AAV-synapsin-GCaMP6m in the central striatum (CS). To visualize individual cell types, KOs were crossed with mice expressing cre-recombinase in D1- or D2-SPNs, and double-transgenic progeny were injected with cre-dependent AAV-GCaMP6m. All mice and WT littermate controls were implanted with 500µm GRIN lenses in the central striatum (CS) to visualize striatal calcium activity during spontaneous grooming behavior. After a baseline imaging session, mice were treated with 5 mg/kg fluoxetine for 7 days and activity of individual SPNs was tracked across sessions.
Sapap3-KO mice displayed elevated calcium activity in the central striatum (combined D1- and D2-SPNs) at the onset of compulsive behavior. This overall increase in activity was driven by an increase in the percentage of cells that were activated at onset of compulsive grooming. Central striatal hyperactivity and numbers of activated SPNs were reduced following treatment with fluoxetine. In contrast activity of D1-SPNs was not elevated at the onset of compulsive behavior in *Sapap3*-KO mice. Instead, D1-SPN activity increased after grooming initiation and showed a sustained elevation throughout a grooming bout, with an increase in the percentage of activated D1-SPNs. Fluoxetine also reduced this sustained D1-SPN activity in *Sapap3*-KO mice. Combined, these data suggest that D2-SPN activity may be strongly increased in *Sapap3*-KO mice at the onset of compulsive behavior.

Disclosures: S.C. Piantadosi: None. **S.E. Ahmari:** None. **B. Chamberlain:** None. **J.R. Hyde:** None. **V.L. Corbit:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.06/Z7

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant F31MH110125

Title: Potentiated transmission of M2 signals in central striatum is related to compulsive grooming behavior

Authors: *V. L. CORBIT[1], S. PIANTADOSI[1], J. WOOD[1], J. CHOI[2], I. B. WITTEN[3], A. H. GITTIS[4], S. AHMARI[1];

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Abstract: Convergent evidence suggests that corticostriatal hyperactivity and hyperconnectivity are associated with Obsessive-Compulsive Disorder and other compulsivity disorders, but it is unknown how these abnormalities lead to compulsive behaviors. In prior work we demonstrated heightened *ex vivo* functional connectivity between anterior M2 cortex (MOs) and central striatum (CS) in a mouse model of compulsive behaviors, the Sapap3-KO mouse (Corbit 2019). In addition, Burguiere et al (2013) demonstrated increased *in vivo* CS activity in SAPAP3-KOs. This suggests that hyperactivity in M2-CS projections may directly lead to CS hyperactivity and compulsive behaviors, consistent with a role for M2 in action monitoring and behavioral selection. However, it is unclear whether this *ex vivo* functional hyperconnectivity,is directly related to striatal hyperactivity and/or compulsive grooming behavior. We therefore investigated the *in vivo* relationship between M2-CS synapses, grooming behavior, and CS activity in SAPAP3-KOs and WT littermate controls. To test the possibility that CS hyperactivity in KOs results from increased upstream M2 basal firing, we performed *in vivo* awake electrophysiological recordings. No genotype differences were observed in baseline M2 firing rates. To assess whether differences in M2 activity could instead be detected during relevant behavioral events, we injected AAV-GCAMP6m in M2, and performed simultaneous fiber photometry in M2 and striatum during grooming tests. Both WTs and KOs showed increased activity in M2 and M2-CS terminals at initiation of grooming bouts. Taken together, these data indicate that differences in M2 activity cannot account for CS hyperactivity in KOs, and suggested the possibility that equivalent drive from M2 results in amplified CS activity in KOs. Consistent with this theory, single-photon calcium imaging using head-mounted *in vivo* microscopes (Inscopix) demonstrated increased CS activity in KOs specifically at the onset of grooming bouts; this heightened activity was due to more grooming-activated cells in KOs. To determine whether increased CS activity could directly cause grooming behavior, we performed bilateral ChR2 stimulation in CS of WT mice. Optogenetic activation of CS evoked partial grooming-related movements in otherwise healthy WT mice. However, specific optogenetic activation of M2-CS terminals evoked full grooming sequences, supporting a key role for M2-CS inputs in the generation of grooming-related hyperactivity. These data together suggest that hyperconnectivity in the M2-CS circuit causes an over-amplification of M2-CS inputs in KOs, leading to compulsive grooming.

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Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.07/Z8

Topic: H.01. Animal Cognition and Behavior

Support: SFB1280

Title: Simple associative learning accounts for an unexpectedly rich dynamics of operant extinction learning

Authors: *J. R. DONOSO[1], Z. LEDERER[1], J. PACKHEISER[2], R. PUSCH[2], O. GÜNTÜRKÜN[2], S. CHENG[1];
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Abstract: Extinction Learning (EL) is the process of changing a previously acquired behaviour as a result of altered reinforcement contingencies, such as the withholding of reinforcements for a previously reinforced behaviour. EL is specific to the context in which it occurred, as evidenced by the so-called renewal of the extinguished behaviour after returning to the original context of acquisition (ABA renewal). Typically, changes in behavior are quantified by comparing post to pre-training blocks. To this end, data are pooled and averaged across many sessions and subjects. Such analysis obscures not only differences across subjects but also changes within single sessions. Here, we report the stunning diversity of learning curves exhibited by pigeons in an operant conditioning task, and offer a simple model that can account for such diversity. Pigeons underwent several sessions, each of which consisted of three phases. In the initial acquisition phase, under context A, animals had to learn to associate two session-unique novel visual stimuli with either a left or a right-peck response, where food was delivered after every correct choice. In the subsequent extinction phase, under either a context B1 or B2, one of the novel stimulus-response associations was no longer rewarded. Once pigeons stopped choosing the associated behaviour, the test phase began with a return to context A, but the extinguished association continued to go unrewarded. Throughout the session, pigeons were additionally presented with two familiar stimuli that underwent no change in their rewarding contingency. Within single sessions, individual learning curves exhibited a wide repertoire of dynamics, expressing several combinations of different features such as persistent selection of unrewarded responses, abrupt transitions of choice upon onset of the extinction context, and absence and reappearance of the renewal effect, among others. These complex behaviours appear to be strategic and to rely on higher-order cognitive functions. However, using a computational model, we show that simple sensorimotor associations and a winner takes all decision process can account for most of the peculiar features observed in the behaviour. The model suggests, that the complexity of behaviour stems from the associative history of context and pecking sides within and across sessions. In conclusion, our work demonstrates how studying the learning dynamics can reveal previously unappreciated nuances in the behaviour and how even simple models can generate complex, apparently purposeful behaviour.

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Poster

085. Learning, Habit, and Compulsion

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Program #/Poster #: 085.08/Z9

Topic: H.01. Animal Cognition and Behavior

Support: NIH RF1 AG017139
NIH R37 AG008796 (JFD)

Title: Acquisition of trace-eyeblink conditioning in female and male C57Bl/6J mice

Authors: *A. P. RAPP, M. OH, C. WEISS, J. F. DISTERHOFT;
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Abstract: It is important to study if and how sex differences affect associative memory because of the potential implications these differences may have for treatment of memory disorders, like Alzheimer's disease. Past research has been largely limited to exclusively male subjects due to prior belief that female subjects would exhibit increased variability due to their estrous cycle. Trace eyeblink conditioning (tEBC) is a forebrain-dependent temporal associative memory task that has been widely used to investigate the mechanisms of associative memory across multiple species, including humans. While past studies (e.g Dalla & Shors, 2009) have shown that female and male rats acquire tEBC at different rates, similar studies have not been performed in mice.
Furthermore, it was recently shown that male and female mice exhibit similar variability in acquiring multiple behavioral tasks, but tEBC was not included in this study (Meziane et al, 2006) Therefore, we examined the role of sex in acquisition of tEBC in a C57Bl/6J mouse model.
To determine if estrogen plays a role in acquisition of associative memory, we have also included both ovariectomized and intact females as separate groups. Our preliminary results suggest that all groups (males, intact females and ovariectomized females) acquire tEBC at similar rates. This discrepancy between our present findings in mice and the past studies in rats, suggests that preclinical testing should be done on multiple animal models. Though rate of acquisition may not differ between the sexes, it is possible regions required for associative memory, like the entorhinal cortex, differ in neuronal firing during acquisition. Future work will investigate this question through in-vivo single-neuron recordings. Understanding the role of sex in associative memory provides a more complete picture of the mechanisms of learning with implications for future targeted therapies.

Disclosures: **A.P. Rapp:** None. **M. Oh:** None. **C. Weiss:** None. **J.F. Disterhoft:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

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Program #/Poster #: 085.09/Z10

Topic: H.01. Animal Cognition and Behavior

Support: NC3Rs Skill and Knowledge Transfer Grant

Title: Open source automated training of non-human primates using facial recognition

Authors: *J. BUTLER, S. W. KENNERLEY;
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Abstract: Traditional laboratory-based training of non-human primates (NHPs) on cognitive tasks is a slow, inefficient process that can cause stress to the animals. To overcome these limitations, we have developed the Mymou system (Greek for "monkey", pronounced my-moo); a fully automated, cheap, open source, home-room training system. The wireless device runs continuously all-day including weekends, allowing NHPs to perform tasks in their home room at their own leisure. The system uses facial recognition to accurately identify each NHP on a trial-by-trial basis, eliminating the need to separate NHPs from their social group while enabling automated individually-tailored. Mymou significantly increases the amount and efficiency of training NHPs complete while also refining the welfare conditions for the animals. Mymou has now been installed in the majority of the UK NHP cognitive neuroscience community (9 labs across Oxford, Cambridge, UCL, and Newcastle Universities). Mymou contains a suite of cognitive tasks that are commonly used in the field of NHP research (e.g. reversal learning, delayed spatial response, associative learning), which have been used to collect a unique dataset from a large and diverse population of NHPs on a variety of standardised cognitive tasks. This is useful at determining the effects of factors, such as gender, age, and diet, on the ability of NHPs to solve various cognitive tasks. This information can therefore aid in improving the effectiveness of an experimenter's training process for their NHP research. As Mymou enables round the clock training, this enabled us to train NHPs on highly advanced cognitive tasks. We devised a novel cognitive task in which NHPs had to learn to navigate networks of associated stimuli to find rewards at certain locations. NHPs engaged with the task on the Mymou system on a daily basis, and were capable of learning a large world consisting of >100 stimuli with nearly 400 individual associations. The NHPs employed sophisticated model-based strategies to solve these tasks. This demonstrates that with the appropriate equipment NHPs can be trained on highly advanced cognitive tasks entirely in their home-room, which will be useful for investigating cognitive behaviours.

Disclosures: J. Butler: None. **S.W. Kennerley:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

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Program #/Poster #: 085.10/Z11

Topic: H.01. Animal Cognition and Behavior

Title: Long-term enriched environment exposure enhances training proficiency in a complex rodent driving task

Authors: *K. G. LAMBERT, D. LESERVE, L. E. CRAWFORD, L. KNOUSE, M. H. KENT, D. VAVRA, K. BREAKALL, C. GLORY, E. MEISEL;
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Abstract: Whereas most rodent laboratory investigations utilize short-term behavioral training protocols with restricted response options, the utilization of a long-term shaping protocol to teach rats to drive a rodent-operated vehicle (ROV) in a forward direction was investigated in the current study. After being exposed to an enriched or control laboratory environment for four months, male Long-Evans rats were habituated to the ROV passenger compartment in their home cages before being shaped to grasp a thin bar to activate the vehicle in a novel driving arena. Subsequently, all rats were shaped to grasp the bar for increased durations to move/drive the car toward the food reward in the test arena. Focusing on the number of training sessions to reach established criteria for each training phase, the enriched animals (n=5) demonstrated mastery of the ROV significantly faster than their control counterparts (n=6) as they more readily explored the car (p=.001), repeatedly grasped the cable for extended durations (p=.02) and reliably drove to the froot loop™ reward (p=.049). Following training, rats were exposed to three extinction sessions. During the second extinction session, enriched rats demonstrated more robust learning in the training trials by entering the car faster than standard housed rats (p=.042). Fecal samples were collected at four time-points during training and extinction to determine effects of training and environment on corticosterone (CORT) and dehydroepiandrosterone (DHEA) metabolites; although no effects of environment were observed, the DHEA/CORT ratios increased with training (p=.001). Thus, the results suggest that the rats' proficiency in this complex task was delayed when housed in standard laboratory conditions. These findings are in agreement with preclinical animal work documenting negative effects of impoverished environments on neural maturation, as well as human research indicating diminished executive functioning in individuals housed in the impoverished conditions of prisons (Meijers et al., 2015). Considering that approximately 11 million individuals across the world live in some form of impoverished detainment (Walmsley, 2013) and about 700 million people live in extreme poverty (World Bank, 2015), additional preclinical research is necessary to provide further information about diminished learning capacity in individuals exposed to impoverished environmental conditions.

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Poster

085. Learning, Habit, and Compulsion

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Program #/Poster #: 085.11/Z12

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH111703

Title: What is the least training a monkey needs to perform an abstract cognitive task?

Authors: ***V. P. FERRERA**[1], G. JENSEN[2], A. MEANEY[1], Y. ALKAN[1], F. MUNOZ[1], H. TERRACE[1];
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Abstract: Rarely is the question asked, "what is the least amount of training needed for an animal to perform a specific task?" Yet, the answer is critical for understanding animals' natural cognitive abilities. Rhesus macaques, trained, for several hundred trials on adjacent items in an ordered list (e.g. A>B, B>C, C>D, etc.), are able to make accurate transitive inferences (TI) about previously unseen pairs (e.g. A>C, B>D, etc). How that learning unfolds during training, however, is not well understood. We sought to measure the relationship between the amount of training and the resulting response accuracy in four rhesus macaques. In particular, we assessed what subjects were able to learn given the absolute minimum amount of training possible on the items in a 7-item list of novel pictorial stimuli. We therefore reduced training to a single presentation of each of the six adjacent pairs prior to testing will all (adjacent and non-adjacent) pairs. Given 6 trials of training and 21 trials of testing (one for each of the 21 possible pairs), subjects only experienced each ordered list for 27 trials. This 27-trial regimen was then repeated with a new set of stimuli. Such brief exposure allowed us to train multiple 7 picture lists per session, providing the power to detect learning even if the effect size was small. We also ran conditions with 24 and 114 trials. In general, learning effects were small, but they varied with roughly the square root of the amount of training. These results suggest that subjects learned serial order in an incremental fashion. Thus, rather than performing transitive inference by a logical process, serial learning in rhesus macaques proceeds in a manner more akin to a statistical inference, with an initial uncertainty about list position that becomes gradually more accurate as evidence accumulates. The relationship between number of training trials and accuracy establishes performance criteria for evaluating computational models of serial learning.

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Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.12/Z13

Topic: H.01. Animal Cognition and Behavior

Title: Rapid and flexible learning of object associations that are context-dependent

Authors: ***M. R. RILEY**, K. L. HOFFMAN;
Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Flexible learning relies on knowing when to apply what rules and associations, such as the context in which informative items appear. In experimental settings, however, we typically measure in isolation either association learning such as paired associates or object-to-place associations, or rule learning, such as rules guiding context-contingent responses. In the present study, we examine within a single experiment both context rules as applied to associated items. In order to further examine this specific context association, we designed a variation of the paired-associate-learning task where associations between cued objects and their corresponding correct targets are altered between different background images that our cue stimulus appears with. Each of the two cues & contexts were associated with a target while three targets were presented during each trial. During training in this task, 2 monkeys rapidly acquired the rules and conditions in only 8 total sessions. Along with improvement in the task percentage-wise, we observed that there was a significant improvement of gaze towards the correct target (regression, p= 0.00017 for context 1, 0.00011 for context 2, and 0.00094 for both contexts presented together). Combined across sessions and contexts, we observed significant differences between percentage of gaze focused on each target type (1-way ANOVA, p=4.52X10-301). A Tukey post-hoc test showed that there was a significantly higher proportion of gaze towards the correct target and significant differences between same-context and opposite-context distractors. Finally, we measured reaction time as a function of learning and observed in each context a significant decrease of RT for training session as well as for correct responses in contrast to same context distractor responses and opposite context distractor responses (2-way ANOVA, context 1 :p=4.3X10-7 for response type and p=0.00033 for session date, context 2: p=2.44X10-6 for response type and p=6.01X10-8 for session date, both contexts: p=6.01x10-16 for response type). These results show a significant effect of context on decision-making when learning paired associate responses indicating that further research into the neural basis of contextual effects on association learning needs to be performed.

Disclosures: **M.R. Riley:** None. **K.L. Hoffman:** None.

Poster

085. Learning, Habit, and Compulsion

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Program #/Poster #: 085.13/Z14

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01-NS088567

Title: Amygdala central nucleus inactivation impairs cerebellar dependent learning in female rats

Authors: *S. R. BRAL[1], S. J. FARLEY[2], J. H. FREEMAN[1];
[1]Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA; [2]Iowa Neurosci. Inst., Iowa City, IA

Abstract: Amygdala central nucleus (CeA) pharmacological or optogenetic inactivation impairs cerebellum-dependent eyeblink conditioning (EBC) in male rats (Farley et al., 2016, 2017, 2018). While previous studies suggest sex differences during EBC following stress (Shors 1998), it is currently unknown if bilateral CeA inactivation in female rats causes similar cerebellar learning impairments as in male rats. In this study, adult female rats underwent delay EBC with either muscimol (GABA-A receptor agonist) or saline infusions into the CeA for the first five training sessions. Non-infusion sessions commenced from session 6 until animals reached an 80% CR criterion. All rats were then subject to a counter-balanced order of saline and muscimol retention sessions. Bilateral CeA inactivation significantly impaired acquisition and retention of EBC. CR percentage during the first session without muscimol for the muscimol group was not different from the first training session for the saline group, indicating no savings in the muscimol group. Both groups reached an equal level of conditioned responses before retention tests. Additionally, both groups were equally impaired during the muscimol retention session. Saline retention revealed the same level of conditioned responses as the criterion session for both groups. These results closely mirror our previous results in adult male rats. Overall, the results indicate that the amygdala's modulatory role in cerebellum-dependent learning is similar for males and females. Funding: R01-NS088567

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Poster

085. Learning, Habit, and Compulsion

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH081153
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Title: Model-free algorithms are inadequate to explain transitive inference performance in monkeys

Authors: *G. G. JENSEN[1,3], H. S. TERRACE[2], V. P. FERRERA[1];
[1]Neurosci., [2]Psychology, Columbia Univ., New York, NY; [3]Psychology, Reed Col., Portland, OR

Abstract: One of the hallmarks of a mature scientific theory is the ability to make specific predictions about a range of experimental observations. For over 100 years, researchers have studied the problem of transitive inference (TI), a widespread ability that tests the ability to understand abstract serial order (e.g. having learned that A>B and B>C, one can infer that A>C). Despite that tradition, there are few quantitative models of serial learning that can account for critical tests of TI performance across multiple experimental paradigms. Furthermore, until very recently, the proposed models have been overwhelmingly derived from model-free reinforcement learning (e.g., variations of the Rescorla-Wagner model). These models assume that TI performance can be explained on the basis of expected value. Due to recent advances in the TI literature, there are now several model-based algorithms for serial learning. We implemented six algorithms (three model-free and three model-based) and found the parameters that best described transitive inference in data collected from rhesus macaques. This allowed us to evaluate not just whether these algorithms were able in principle solve certain TI problems, but also whether they solved them in the same manner as monkeys. We then used those parameters to simulate performance under a range of experimental manipulations, allowing, for the first time, a comparison of all six models on a level playing field. Although no algorithm provided an entirely satisfactory account of all behaviors observed in the experimental literature, model-based approaches (which deduced serial order by representing stimuli long a continuum) predicted overall performance better than model-free approaches. These results argue against the idea that TI performance is explained by the comparison of expected values.

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Poster

085. Learning, Habit, and Compulsion

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Program #/Poster #: 085.15/Z16

Topic: H.01. Animal Cognition and Behavior

Support: R01-NS088567

Title: Optogenetic inhibition of amygdala central nucleus efferent pathways modulate cerebellum dependent learning

Authors: *S. J. FARLEY[1,2], J. H. FREEMAN[1,2];
[1]Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA; [2]Iowa Neurosci. Inst., Iowa City, IA

Abstract: Amygdala output is known to play a modulatory role in cerebellum-dependent learning (Farley 2016, 2017, 2018). Pharmacological inhibition of the amygdala central nucleus (CeA) results in a reliable impairment acquisition and retention of the cerebellum-dependent delay eyeblink conditioning (dEBC) in adult rats. Further, optogenetic inhibition of CeA neurons that is temporally synced with the onset of the conditioned stimulus (2KHz tone) impairs acquisition (Farley 2018). It is unknown if amygdalar modulation of the cerebellum acts through one or multiple CeA efferent pathways. In this study, we optogenetically targeted three efferent pathways of the rat CeA that may modulate cerebellar dependent learning. An inhibitory optogenetic vector (AAV5-hSyn-eArch3.0-EYFP) was bilaterally delivered to the CeA. Five weeks after viral injection optical fibers were placed in CeA neuron terminal fields of one of three projection targets: basilar pontine (PN), locus coeruleus (LC), or ventral lateral periaqueductal grey (vlPAG). Photo-stimulation with a 561 nm laser was limited to the duration of the CS only. Controls animals were injected with an opsin-lacking AAV (AAV5-hSyn-EYFP). After recovering from optical implant surgery, rats commenced training in five, 100-trial sessions of dEBC. Trials consisted of paired tone (conditioned stimulus [CS]) and peri-orbital shock (unconditioned stimulus [US]). Optogenetic inhibition of the targeted CeA efferent pathways revealed differential effects on acquisition of dEBC. Inhibition during the CS period of the CeA—PN projection or the CeA—LC projection impaired the rate of conditioned responses (CRs). However, CRs were not impaired in animals receiving inhibition of the CeA—vlPAG projection or the control group. Conversely, the amplitude of the CR was impaired for animals receiving inhibition of the CeA—vlPAG projection, yet inhibiting the CeA—PN projection or the CeA—LC projection did not impair CR amplitude. Inhibiting the CeA—vlPAG projection or the CeA—LC projection reduced the amplitude of the unconditioned response relative to controls, but was not impaired inhibiting the CeA—PN projection. These results suggest CeA efferent projections contribute to different aspects of cerebellum-dependent associative learning.

Disclosures: S.J. Farley: None. **J.H. Freeman:** None.

Poster

085. Learning, Habit, and Compulsion

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ERC StG MEMCIRCUIT

Title: Strategies for optimized design and behavioral training of cognitive tasks in head-fixed mice

Authors: *T. W. BERNKLAU, L. S. MEHRKE, D. HÄHNKE, A. RANGANATH, S. N. JACOB;
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Abstract: In order to isolate and study specific cognitive components of higher brain functions, behavioral tasks must be well controlled. Mice are gaining importance as a model system in cognitive neuroscience research because of the growing number of techniques to specifically measure and manipulate brain activity. Yet, due to their limited cognitive abilities, behavioral protocols for probing higher cognitive functions in mice are rare and mostly relying on simpler, often innate behaviors. Head-fixed preparations, highly advantageous to achieve a controlled sensory environment and to access the entire range of experimental tools, further restrict the behavioral repertoire. We found that adherence to specific principles in task design and behavioral training, which are derived from characteristics of the mouse animal model, greatly facilitate learning of tasks that are cognitively challenging for head-fixed mice. We trained a variety of goal-directed behaviors requiring instrumental learning and abstract sensory-motor integration, using different response devices with either continuous readout (turning a wheel) or binary readout (licking spouts) and stimuli from different sensory modalities (auditory and visual). We obtained three main results regarding specifics of the animal, task design and training strategies. First, a protocol based on largely automated procedures including means to control response bias and a consistent daily schedule can reduce the behavioral variability within and across animals to a level comparable to non-human primates, counteracting the typical sensitivity to marginal sensory changes seen in mice. Second, tasks that encourage the animal to actively engage with its environment produce strong learning. This can be achieved for example by closed-loop sensory feedback for motor actions. Third, training requiring minimal strategy changes of the animal and the introduction of "no-choice" trials guiding the correct response at early learning stages create facilitative conditions that keep the animal motivated and result in constant levels of high performance and rapid learning. The potential of adhering to these principles is illustrated in an auditory-guided licking task, for which optimization reduced

training time by two- to three-fold. Our results demonstrate a modus operandi for behavioral training of head-fixed mice and could serve to encourage the development of more sophisticated tasks for the in-depth investigation of the neuronal mechanisms of higher cognition.

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Poster

085. Learning, Habit, and Compulsion

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Topic: H.01. Animal Cognition and Behavior

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Title: The Janelia Smart Cage: A system for automated training of head-restrained mice in their home cage

Authors: *N.-W. TIEN[1,2], H. INAGAKI[1], M. INAGAKI[1], J. ARNOLD[1], P. POLIDORO[1], S. DRUCKMANN[1,2], A. LEONARDO[1], K. SVOBODA[1];
[1]Janelia Res. Campus, HHMI, Ashburn, VA; [2]Dept. of Neurobio., Stanford Univ., Stanford, CA

Abstract: Head-restrained animals are routinely trained on behavioral tasks designed to isolate specific sensory, cognitive or motor functions. Head-fixation provides stimulus control and behavioral read-out, and thus has been the foundation on which many conceptually rich and quantitative studies of the neural basis of behavior have been built.
Training head-restrained behaviors is typically supervised by experts, who adjust task parameters in real time to shape learning. This process is time-consuming. Undocumented differences in training procedures across trainers and labs can lead animals to develop different strategies to solve the same task. We have developed a system, the Janelia Smart Cage (JSC), for automated training of head-restrained mouse behavior. The goal is to facilitate highly parallelized and standardized training in the home cage. Self-initiated training may also be less stressful for mice to learn complex cognitive tasks.
We modified a mouse cage (size, 18.6 x 29.8 x 12.8 cm) that can be mounted into a standard rack. Some features were inspired by the system described by Murphy *et al* (*Nat Comm*, 2016). Mice were implanted with a custom headbar. They receive all of their water via a pair of lickports mounted on the front of the cage. Access to the lickports is through a tapered tunnel. Grooves in the tunnel constrain head movements to one dimension. Latches powered by stepper motors fix the headbar with high repeatability.
We trained mice in an auditory delayed-response task (Inagaki *et al* JNS 2018). Mice report their

decision by directional licking following a delay epoch. Mice performed > 700 trials per session and reached above 70 % performance within 4 weeks, comparable to manual training. After transfer to recording rigs mice continued to perform at a high level. Lesioning anterior lateral motor cortex in well-trained mice dropped behavior to near chance level and behavioral performance did not recover. The JSC is based on low-cost, commodity components and a few 3d printed custom parts and is therefore easily cloned. The JSC provides a platform for efficient and high-throughput behavioral training, quantitative comparisons of diverse training procedures, and in-cage optogenetic experiments.

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Poster

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Title: Autonomous mouse behavioral testing and loss-of-function screen in homecage with voluntary head-fixation

Authors: Y. HAO, *N. LI;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Behavior is orchestrated by activity distributed over multiple brain regions. Yet for most behaviors, we do not know comprehensively which brain areas are involved and when each region contributes. Inactivating individual brain regions can assess their involvements in behavior. However, the large number of brain regions to be tested and experimental throughput limit comprehensive surveys. To overcome this limitation, we developed an automated system for head-fixation, behavioral training, and optogenetic testing. Mice can be trained to perform decision-making tasks and undergo loss-of-function screens in their homecages without human supervision.
Naïve mice implanted with a headbar are lured by a motorized lickspout into a head-fixation port

coupled to their homecage. A computer program gradually acclimates the mice to head restraints. Mice can self-release at any time by pressing against a load-sensing floor. Next, another computer program teaches the mice to perform decision-making tasks. After the mice reach high levels of performance, a red (635nm) laser is used to broadly illuminate the brain through a clear skull preparation, stimulating optogenetic opsins selectively expressed in targeted brain region(s). The whole process, controlled by microcontrollers, runs autonomously 24/7 without any human supervision.
Mice successfully learned a tactile decision-making task in the automated system. Mice usually performed over 800 trials in one day. The training time to reach criterial performance was comparable with manual training. Mice trained in the automated homecage system retained high-levels of task performance when transferred to separate electrophysiology or imaging rigs, allowing for neurophysiology experiments. We used a red-shifted channelrhodopsin, ChrimsonR, expressed in GABAergic neurons to photoinhibit activity in several known brain regions involved in the tactile decision behavior, including anterior lateral motor cortex and somatosensory cortex. Photoinhibition during the automated homecage behavior elicited the same patterns of behavioral deficit as those observed in manual experiments (Guo et al. Neuron, 2014).
The whole system, including optogenetics components, costs <$3,000. Parallelizing multiple cages can test many brain regions across a large number of mice. The system follows a modular design for head-fixation, behavioral task, optogenetics, and therefore can be adapted for a wide range of use cases. The approach has the potential to drastically increase the throughput of loss-of-function experiments and comprehensively map brain networks involved in behavior.

Disclosures: **Y. Hao:** None. **N. Li:** None.

Poster

085. Learning, Habit, and Compulsion

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 085.19/Z20

Topic: H.01. Animal Cognition and Behavior

Support: National Natural Science Foundation of China [grant number 81674057]

Title: Effects of acupuncture on behavioral stereotypies and brain dopamine system in mice as a model of Tourette syndrome

Authors: *L. LIN, M. LI, H. XIANG, X. HU;
Tong Ji Med. Col., Wuhan, China

Abstract: Tourette syndrome (TS) is a developmental neurobehavioral disorder characterized by involuntary behavioral stereotypies. Clinical studies have confirmed the positive effect of

acupuncture on treating TS, but the underlying mechanisms are not fully understood. In the present study, we used behavioral tests, western blotting, double-immunofluorescence labeling and fluorescence spectrophotometry to investigate whether acupuncture performed at acupoints "Baihui" (GV20) and "Yintang" (GV29) affected behavioral stereotypies and regulated the dopamine (DA) system in three different brain regions in Balb/c mice injected with 3, 3'-Iminodipropionitrile (IDPN) as model for TS. We found that acupuncture alleviated behavioral stereotypies, down-regulated the expression of D1R and D2R in striatum and substantia nigra pars compacta (SNc), as well decreased the concentration of DA in striatum, SNc and prefrontal cortex (PFC). Moreover, acupuncture also reduced the expression of tyrosine hydroxylase (TH) in SNc. Conclusively, acupuncture ameliorated behavioral stereotypies through regulating DA system in striatum, SNc and PFC. Our findings provide novel evidence for the therapeutic effect of acupuncture on TS.

Disclosures: L. Lin: None. **M. Li:** None. **H. Xiang:** None. **X. Hu:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.01/Z21

Topic: H.01. Animal Cognition and Behavior

Support: JST ERATO (JPMJER1801)
JSPS Grants-in-Aid for Scientific Research (18H05525)
the Human Frontier Science Program (RGP0019/2016)

Title: Hilar mossy cells encode hippocampal ripple information

Authors: *A. OUCHI, M. SATO, Y. IKEGAYA;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Hippocampal sharp waves / ripples (SPW-Rs) are high-frequency oscillations emitted mainly during slow-wave sleep or quiet rest states and play a key role in memory consolidation. While SPW-Rs are initiated in the CA3 subregion and propagate to the downstream CA1 subregion, our previous study demonstrates that they also propagate back to the dentate gyrus in acute brain slice preparations. However, neither the role of CA3-to-DG SPW-Rs backpropagation nor its propagation mechanism has been fully understood. Furthermore, it is hard to verify SPW-Rs backpropagation in *in vivo* experiment because the CA3 subregion and the dentate gyrus are located deep in the hippocampus. We previously demonstrated that the combinatorial dynamics of the membrane potentials of three hilar mossy cells represent the activity structures (waveform features) of SPW-Rs using information theoretical analysis. We thus concluded the information encoded by CA3 pyramidal cells is somehow compressed into a

subset of mossy cells; note that mossy cells are numerically less dominant than hippocampal principal neurons, such as pyramidal cells or granule cells. Therefore, we hypothesize that mossy cells may be hub neurons of SPW-Rs backpropagation. To verify the consistency between *in vivo* and *in vitro*, we conducted *in vivo* whole-cell recordings from mossy cells together with recordings of ripple oscillations from the CA1 subregion in urethane anesthetized mice. We have so far succeeded current-clamp recordings from 9 mossy cells and suggested that mossy cells are responsive to ripples. Our research approaches further elucidation of brain information dynamics and will provide a new perspective to an information processing mechanism.

Disclosures: A. Ouchi: None. **M. Sato:** None. **Y. Ikegaya:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.02/Z22

Topic: H.01. Animal Cognition and Behavior

Support: the LouLou Foundation
Intellectual and Developmental Disability Research Center (U54 HD083092), NIH Grant R01 NS100738

Title: Hippocampal neural circuit dysfunction contributes to memory deficits in CDKL5 mutant mice

Authors: *S. HAO[1,2], Q. WANG[1,2], B. TANG[1,2], Z. WU[1,2], J. TANG[1,2];
[1]Dept. of Pediatrics, Baylor Col. of Medici, Houston, TX; [2]Jan and Dan Duncan Neurolog. Res. Institute, Texas Children's Hosp., Houston, TX

Abstract: Mutations in the X-linked gene cyclin-dependent kinase-like 5 (*CDKL5*) cause CDKL5 disorder, a neurodevelopmental disease characterized by early-onset seizures, severe intellectual disability, motor impairment, and autistic features. Some of the symptoms such as hippocampus-dependent memory deficits were also observed in animal models loss of CDKL5. However, how CDKL5 absence shapes the hippocampal neural circuit activity and, subsequently, affects the related memory function has not been elucidated. Here we first assessed the hippocampus-dependent learning and memory in a unique CDKL5 knockout mouse model with exon 6 deletion (B6.129(FVB)-*Cdkl5*$^{tm1.1Joez}$/J). Compared to the wide type (WT, *Cdkl5*$^{+/y}$) littermates, the CDKL5 null mice (*Cdkl5*$^{-/y}$) exhibited significantly impaired performance in hippocampus-dependent memory tasks including fear conditioning, passive avoidance, and Morris water maze. In parallel, long-term potentiation in the perforant path (PP) to the dentate gyrus (DG) pathway was impaired in awake, freely moving *Cdkl5*$^{-/y}$ mice when tested at 1 h, 1 day, and 2 days after induction, compared with WT controls. Meanwhile, our *in vivo* testing in

freely moving *Cdkl5*$^{-/y}$ mutant mice revealed an increase of feedforward inhibition in the PP-DG pathway, suggesting the influence of CDKL5 over local inhibitory circuits within the DG. It has been established that the molecular layer perforant pathway (MOPP) cells located in the molecular layer of DG contribute to feedforward inhibition of DG via PP activation. Therefore, we further investigated the local hippocampal synaptic input in MOPP and granule cells in brain slices. Ablation of CDKL5 enhanced the excitatory inputs to the MOPP cells, and consequently, resulted in increased inhibitory input to the DG granule cells. Moreover, DG granule cells from *Cdkl5*$^{-/y}$ mice showed a reduced excitatory input received directly from PP, indicating that CDKL5 loss caused a shift in the balance between excitation and inhibition, which is crucial for maintaining normal brain functions. Together, our findings demonstrate that CDKL5 mediates hippocampal learning and memory likely through the regulation of DG circuitry activities. This work was supported by the LouLou Foundation, the Intellectual and Developmental Disability Research Center (U54 HD083092), and 5 R01 NS100738.

Disclosures: S. Hao: None. **Q. Wang:** None. **B. Tang:** None. **Z. Wu:** None. **J. Tang:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.03/Z23

Topic: H.01. Animal Cognition and Behavior

Title: Microglia mediate dissociation of memory engrams and forgetting via complement-dependent synapse elimination

Authors: *C. WANG[1,2], Y. GU[1,2];
[1]Ctr. for Stem Cells and Regenerative Medicine, Zhejiang Univ. Sch. of Medicine, Hangzhou, China, Hangzhou, China; [2]Ctr. for Neuroscience, Zhejiang Univ. Sch. of Medicine, Hangzhou, China, Hangzhou, China

Abstract: Memory is coded and allocated to engrams within related brain regions. Synaptic connections between engram cells are believed to be substrates for memory storage, weakening or loss of these synapses leads to failure in retrieval of encoded memory information. Microglia are not only important for pruning excessive synapses during postnatal brain development, but also highly involved in the dynamics of synapses even in the adult brain. However, it remains unclear whether and how microglia are involved in memory forgetting. We found in the healthy adult hippocampus, microglia retain the ability to engulf synaptic structures via phagocytosis. Temporal elimination of microglia prevents memory forgetting and engram dissociation. Specific inhibition of complement cascades within engram cells prevents forgetting of related memory. Moreover, we found complement-dependent synaptic elimination by microglia is involved in not only neurogenesis-mediated forgetting, but also non-neurogenesis-mediated

forgetting. Our study demonstrates that complement-dependent elimination of synapses by microglia mediates memory forgetting in the healthy adult brain.

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

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.04/Z24

Topic: H.01. Animal Cognition and Behavior

Support: NIH RF1 AG017139
NIH R37 AG008796 (JFD)

Title: Genetic ablation of neural progenitors in mice impairs acquisition of trace eyeblink conditioning

Authors: ***L. N. MILLER**, C. WEISS, J. F. DISTERHOFT;
Northwestern Univ., Chicago, IL

Abstract: New neurons born in the adult brain are highly excitable and are believed to play a role in memory formation by providing enhanced plasticity to the hippocampus. Past studies have demonstrated that ablation of these adult-born neurons impairs acquisition of various associative memory tasks, but these experiments often made use of irradiation or neurotoxic substances, which may have led to unintended off-target effects. Therefore, to investigate the role of these neural progenitor cells more precisely, we utilized C57BL/6-Tg(Nes-TK*,-EGFP)145Sker transgenic mice (Nes-TK) to selectively reduce the number of newborn neurons in both male and female mice. Nes-TK mice were fed a chow infused with valganciclovir (Val), a pro-drug of ganciclovir, to induce the ablation of neural progenitors. Control groups consisted of wildtype mice on Val-chow, wildtype mice on regular chow, and transgenic mice on regular chow. After being on their respective diets for four weeks, animals were trained on trace eyeblink conditioning (tEBC), a hippocampal-dependent temporal associative memory task, for a total of ten days. Following the completion of training, brain sections from these animals were stained for doublecortin (DCX), a marker for immature neurons, to quantify levels of neurogenesis. We found that male transgenic mice on Val (n=12) had significantly decreased amounts of DCX relative to male control animals (n=11), indicating a successful reduction in levels of neurogenesis. In conjunction with this reduction in neurogenesis, the transgenic male mice on Val learned at a slower rate than male control mice. Female transgenic mice on Val (n=5), however, did not have significantly lower DCX levels than control mice (n=6) and performed at similar levels as control females on tEBC. Interestingly, we observed no difference in the rate of learning between male and female control mice, in contrast to previous studies that

found that female rats learn tEBC faster than male rats. Apart from this lack of sex difference, these results are consistent with prior studies that demonstrated that adult-born neurons are involved in the formation of associative memories. This experiment also provides a foundation to further explore the physiological role of these neural progenitors and other cell types in dentate gyrus through *in vivo* recordings during behavioral training.

Disclosures: L.N. Miller: None. **C. Weiss:** None. **J.F. Disterhoft:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.05/Z25

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01NS093866

Title: Granule cells of the dentate gyrus active in a novel environment are regulated by unique inhibitory circuitry

Authors: *W.-C. SHU, Y. MA, M. B. JACKSON;
Neurosci., Univ. of Wisconsin-Madison, Madison, WI

Abstract: The Cre-recombinase dependent hVoS 1.5 reporter mouse line can target the hybrid genetically-encoded voltage sensor hVoS1.5 to distinct types of cells, thus providing a tool for the study of complex patterns of activity in neural circuits as they encode, process, and store information. To investigate how neural circuitry controls firing patterns in dentate granule cells (GCs), we targeted hVoS probe to different subsets of GCs and imaged their electrical activity. The c-fos-Cre-ER mouse was crossed with the hVoS reporter mouse to target cells active during specific behaviors. We then placed these mice in a novel environment to induce hVoS probe expression in neurons activated by novelty. Control labeling of GCs was achieved with mice generated by crossing the Prox1-Cre-ER mouse with the hVoS reporter mouse. In these mice, mature GCs can be randomly labeled at a low density. We used hVoS imaging to image electrical responses of GCs to stimulation of the perforant path, and compare the responses between these two sets of neurons. We focused our attention on neurons that produced double-spike responses as this serves as an indicator of circuit function. Both populations of GCs can produce a double-spike response with two peaks separated by an interval of 4-5 msec. Since GABAergic inhibition controls GC firing, we tested the role of $GABA_A$ receptors in double spikes. hVoS imaging of control GCs (labeled with Prox1) showed that double-spike responses occur more often following $GABA_A$-receptor blockade. However, in GCs labeled with c-fos-Cre in a novel environment, $GABA_A$-receptor blockade resulted in multiple spikes with an interval of ~10 msec rather than just double spikes. These data suggest that GCs that respond to novelty are

more strongly regulated by GABAergic inhibition. These circuit properties may be relevant to how the dentate gyrus processes novel information.

Disclosures: W. Shu: None. **Y. Ma:** None. **M.B. Jackson:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.06/Z26

Topic: H.01. Animal Cognition and Behavior

Support: NIH IRP ZIAMH00274

Title: The role of adult neurogenesis ablation in a probabilistic learning task

Authors: *K. B. HUNTZICKER[1], R.-M. KARLSSON[2], H. A. CAMERON[3];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]NIMH, Natl. Inst. of Hlth. (NIH), Bethesda, MD; [3]NIMH, NIH, Bethesda, MD

Abstract: Hippocampal adult neurogenesis is implicated in the neural mechanisms for many types of complex learning. In this study, we employ a time- and region-specific pharmacogenetic model of neurogenesis ablation to study the role of recently-born hippocampal neurons in probabilistic learning without subjecting animals to injection or surgical stress. Rats express herpes simplex virus thymidine kinase (HSV-TK) under the control of the human glial fibrillary acidic protein (GFAP) promoter. In these animals, the oral administration of valganciclovir triggers successful ablation of hippocampal neurogenesis. Treated GFAP-TK (TK) rats do not exhibit any deficits in many standard learning tasks; however, previous studies from our lab have found that TK rats show differential responses to ambiguous threat cues when compared to wild-type controls. We propose that this effect could extend beyond threat cues: that TK rats may behave differently in response to ambiguous feedback in an operant probabilistic learning paradigm. Rats will learn that one of two levers produces a food reward pellet 80 percent of the time, and the other lever, 20 percent. This probabilistic learning -- and subsequent reversal learning when lever outcomes are switched -- can be measured and interpreted with Bayesian analyses. In such a task, rats frequently encounter ambiguous feedback, as levers do not always produce the expected outcome, and unexpected outcomes can signal either a reversal or merely the 20 percent chance that a correct lever produces no reward. The data collected will allow analysis of cognitive flexibility as well as the behavioral response to both positive and negative feedback. By using outcome certainties that are probabilistic rather than deterministic, the paradigm is more translatable to human learning studies, in which probabilistic reward schedules are frequently used to slow learning. This paradigm is also more representative of situations in nature - rarely do animals ever encounter a cue that is perfectly predictive. The results from this

study will allow us to determine whether TK rats indeed show differential responses to unexpected wins and losses, therefore suggesting a role for adult neurogenesis in probabilistic learning.

Disclosures: K.B. Huntzicker: None. **R. Karlsson:** None. **H.A. Cameron:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: MOST Grant 106-2320-B-010 -011 -MY3
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MOST Grant 108-2923-B-010-001 -MY2
MOST Grant 108-2321-B-010-001-
NHRI Grant NHRI-EX108-10814NI

Title: Long-range GABAergic projections connect the dentate gyri of the two hemispheres in a cell type-specific fashion and support contextual memory

Authors: T.-Y. YEN[1,2], M. I. SCHLESIGER[3], D. A. MACLAREN[4], C.-C. LIEN[1], H. MONYER[5];
[1]Natl. Yang-Ming Univ., Brain Res. Ctr., Taipei, Taiwan; [2]Academia Sinica and Natl. Yang-Ming Univ., Taiwan Intl. Grad. Program in Mol. Med., Taipei, Taiwan; [3]Dept. of Clin. Neurobio., [4]Med. Fac. of Heidelberg Univ. and DKFZ, Heidelberg, Germany; [5]Clin. Neurobio. A230, Med. Fac. of Univ. Heidelberg & DKFZ, Heidelberg, Germany

Abstract: The dentate gyri are connected inter-hemispherically by long-range-projecting GABAergic neurons. However, the connectivity and function of this projection remains unknown. Using optogenetically-assisted functional mapping, we established *in vitro* that SOM^+ contralateral-DG (cDG) projections form functional inhibitory synapses on inhibitory, non-fast-spiking neurons as well as on a small proportion of excitatory cells. In behaving mice, directly targeted neurons were identified as predominantly non-spatial,(putative interneurons), and accordingly other neurons in the network were disinhibited..Unilateral optogenetic stimulation of SOM^+ cDG terminals disrupted performance in a conditioned place preference task and resulted in an increase in the number of $cFos^+$ cells in the stimulated hemisphere. This increase was not seen after stimulation during the exploration of a non-conditioned context. Taken together, we probe the functional connectivity between cDG long-range-projecting GABAergic neurons and their distal target neurons and provide evidence for a role of these projections in a DG-dependent memory task.

Disclosures: **T. Yen:** None. **M.I. Schlesiger:** None. **D.A. Maclaren:** None. **C. Lien:** None. **H. Monyer:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.08/Z28

Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01 MH108623
NIMH R01 MH111754
NIMH R01 MH117961
IMHRO/One Mind

Title: Experience-dependent enhancement of odor representations in the dentate gyrus

Authors: N. I. WOODS[1], F. STEFANINI[3], D. APODACA-MONTANO[1], I. TAN[1], J. BIANE[2], *M. KHEIRBEK[4];
[2]Psychiatry, [1]Univ. of California San Francisco, San Francisco, CA; [3]Columbia Univ., New York, NY; [4]Univ. of California, San Francisco, San Francisco, CA

Abstract: Animals use cues in their environments to guide behavior, and with experience, modify internal representations of these cues to guide future behavior. How external stimuli are transformed into patterns of neural activity that are modified by experience remains a central question in neuroscience. Using high-resolution calcium imaging of hippocampal activity, we find representations for olfactory stimuli in the dentate gyrus (DG) subregion of the HPC that are enhanced with learning. The degree to which odorants were discriminated by patterns of activity in DG granule cells (GCs) was directly related to future olfactory learning across animals. With learning, DG GCs transformed highly overlapping odor representations in lateral entorhinal cortex (LEC) into sparse, non-overlapping patterns in the DG that enhanced odor representations, providing real-time evidence of the DG performing pattern separation on cortical input. These data reveal that DG GCs are a crucial node of the extended network that represents the olfactory world and for learning the associations between olfactory stimuli and behaviorally relevant outcomes.

Disclosures: **N.I. Woods:** None. **M. Kheirbek:** None. **F. Stefanini:** None. **D. Apodaca-Montano:** None. **I. Tan:** None. **J. Biane:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.09/Z29

Topic: H.01. Animal Cognition and Behavior

Support: T32 MH01574
R37 MH068542
R01 MH083862
R01 AG043688
R01 NS081203
HDRF RGA-13-003

Title: Dissecting the neural circuit for discrete cue representation in the dentate gyrus

Authors: *S. N. TUNCDEMIR[1], C. O. LACEFIELD[1], G. TURI[1], R. HEN[2];
[1]Dept. of Psychiatry, Columbia University/ RFMH, New York, NY; [2]Neurosci. and Psychiatry, Columbia Univ., New York, NY

Abstract: Discrimination of different contexts composed of distinct constellations of multisensory cues is a hallmark of both episodic memory and spatial navigation, two functions ascribed to the mammalian hippocampus. The dentate gyrus of the hippocampus is central to spatial and contextual discrimination; yet the neural mechanisms by which contextual representations are encoded by principal granule cells has remained a significant knowledge gap. To this end, we employed *in vivo* two-photon calcium imaging during behaviors designed to allow precise control over task variables such as the cue and reward locations. We have designed and implemented an adult hippocampal neurogenesis-dependent goal-oriented learning task where mice learn a new reward location as they run repeatedly through a fixed sequence of tactile cues on a treadmill. By imaging large populations of dentate gyrus neurons, we discovered a novel subset of granule cells, which form a robust representation of specific cue locations that persist through learning. Both cue-associated activity and the rate of learning are reduced in mice without adult hippocampal neurogenesis. Critically, these granule cells rapidly respond to cues of different modalities and track cues across locations. Thus, we hypothesize that specialized cue cells in the dentate gyrus are critical for anchoring contextual representations and are modulated by adult born granule cells. Since hippocampal damage has been implicated in the cognitive discrimination impairments associated with Alzheimer's disease and PTSD, constructing a dynamic picture of the dentate gyrus during the formation of episodic memories may have an important clinical relevance.

Disclosures: S.N. Tuncdemir: None. **C.O. Lacefield:** None. **G. Turi:** None. **R. Hen:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.10/Z30

Topic: H.01. Animal Cognition and Behavior

Support: German Research Foundation-FOR2143
ERC-AdG787450

Title: Single unit recordings of somatostatin-expressing interneuron types in mouse dentate gyrus exposed to a virtual reality

Authors: *M. YUAN[1,2], M. BARTOS[1];
[1]Inst. For Physiol. I, Freiburg, Germany; [2]Fac. of Biol., Freiburg, Germany

Abstract: The dentate gyrus (DG) as the input gate of the hippocampus receives a rich multimodal input about space and events from the entorhinal cortex via the perforant path and translates this rich input stream into sparse non-overlapping memories. By decorrelating the rich input stream into non-overlapping sparse memories, the DG is hypothesized to allow a high segregation and sparsification of information. This information is further transmitted from the DG via the classical tri-synaptic path through the hippocampal area CA3 to CA1 and forwarded to cortical networks for long-term storage after consolidation. The DG shows an enormously sparse activity compared to other brain areas. Indeed, neuronal activity in the DG stays under tight inhibitory control indicating that encoding of information is controlled by synaptic inhibition. Among the various GABAergic interneuron types, somatostatin-expressing interneurons (SOMIs) form a large proportion of the DG interneuron population. However, little is known on the activity of DG-SOMIs and their contribution to sparse DG activity. Here we used single unit recordings with 4-shank silicon probes (64 channels) in head fixed mice exposed to a virtual reality (VR) to record optogenetically identified SOMIs. Mice were trained to perform a goal-oriented learning task on a circular track in the VR. Our previous work showed that DG-SOMIs fall into at least two different types, one with axonal arbors in the molecular layer and the other one with axons in the hilus projecting to the medial septum (Yuan et al., eLife 2017). Our current data show that channelrhodopsin-2-expressing SOMIs can be reliably identified by light application (470 nm, 50 ms) to the DG and to the fornix thereby identifying local vs septal projecting DG-SOMIs. Their activity is strongly theta frequency (4-12 Hz) but only mildly gamma frequency (30-120 Hz) modulated. Interestingly, in contrast to fast-spiking interneurons, SOMI discharges are negatively correlated with running speed. In summary, we provide first data onto the activity of different SOMI types in the DG of mice exposed to the VR.

Disclosures: **M. Yuan:** None. **M. Bartos:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.11/Z31

Topic: H.01. Animal Cognition and Behavior

Support: NIH R03MH110749
FSU

Title: GLP-1R activation in motivation and hippocampal-dependent cognitive function

Authors: *D. L. GRAHAM, N. HENDERSON, H. MADKOUR, T. TRAMMELL, G. D. STANWOOD;
Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: Major depressive disorder (MDD) is a common neuropsychiatric disorder characterized not only by negative affect but also with cognitive impairments. Typical antidepressant pharmacotherapies treat the mood symptoms, but oftentimes the memory deficits remain. The glucagon-like peptide-1 receptor (GLP-1R) is a known modulator of many physiological processes, including energy homeostasis, food intake, and drug reward. Activation of this receptor has also been shown to enhance working memory and may have antidepressant-like properties. We and others have shown that GLP-1R is highly expressed in several key brain regions associated with these outcomes, including the dentate gyrus of the hippocampus. This brain region modulates the cognitive and mood-related outcomes associated with MDD. GLP-1R agonists are currently approved for treatment of type 2 diabetes and obesity and are under evaluation for a number of disorders, including Parkinson's and Alzheimer's diseases. As pharmacotherapies to treat deficits in MDD-associated cognitive deficits are non-existent, we hypothesized that GLP-1R activation would enhance motivation and learning and memory. We tested male and female C57Bl/6J wild-type mice in a progressive ratio task using Bussey-Saksida-style operant chambers. Following training, mice were acutely administered a GLP-1R agonist, exendin-4 (Ex-4), or saline (ip). Ex-4 treatment altered performance (i.e., breakpoint) relative to vehicle administration. We then investigated whether GLP-1R activation would alter location discrimination, a cognitive domain that is affected by MDD in humans. Mice were trained in a location discrimination task in the operant chambers and tested in probe trials that utilized easy and difficult parameters. Prior to each probe trial, mice were administered Ex-4, either peripherally or centrally (icv or dentate gyrus), or a vehicle control. GLP-1R activation enhanced cognitive performance in the LD task in a sex-dependent manner. These data indicate that GLP-1Rs are potential targets to treat cognitive impairments associated with MDD. However, GLP-1R's role in the motivational and mood aspects of MDD necessitates further study. Funded by R03MH110749 (DLG) and FSU.

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Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.12/Z32

Topic: H.01. Animal Cognition and Behavior

Support: DFG: SFB 936

Title: Reactivation of neuronal ensembles in the dentate gyrus is required to update spatial memories

Authors: *P. J. LAMOTHE-MOLINA[1], A. FRANZELIN[1], L. M. AUKSUTAT[2], F. MORELLINI[2], T. G. OERTNER[1];
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Abstract: Navigation in complex environments is fundamental for survival. In mammals, this process involves the hippocampal formation. For goal-directed navigation, it is necessary to compare the current and the target position using an internal representation of space, the cognitive map. Since real-life environments are constantly changing and unpredictable, cognitive flexibility and pattern separation abilities are critical for adaptive behavior. The dentate gyrus is involved in both of these processes; therefore, we sought to address the neuronal ensembles dynamics during spatial memory formation and reversal learning. We used c-Fos-driven neuronal tagging (TetTag: cFos::shEGFP/cFos::tTA) to identify two highly active granule cells (GC) ensembles on two consecutive days while mice were trained to locate the position of a hidden platform in the Morris water maze. We analyzed *ex vivo* brain slices (40 µm) using immunohistochemistry and confocal microscopy. Images were obtained from both dentate gyri from 6 different slices along the antero-posterior axis, covering the whole dorsal hippocampus. Reactivation of neuronal ensembles was reported as overlap/chance. First, we found that GC ensemble activity changed considerably on two consecutive days in the home cage. The overlap was even lower than chance level, indicating that the system actively avoided using the same GCs on consecutive days. During early learning in the water maze (day 1 vs. day 2), we found a high degree of overlap between day-to-day activity maps, but not during the late training phase (day 5 vs. day 6). However, we observed a high degree of overlap when the late training phase was followed by reversal learning (i.e., learning that the he platform was located at a new position in the water maze). This observed high overlap was not due to environmental novelty, since mice that experienced water maze training on day 1 and open field (different experimental room) on day 2 showed extremely low overlap. We next delivered a chloride-conducting

channelrhodopsin (iChloC) to selectively silence the GCs that were most active during the first day of water maze training. Consistent with our overlap quantification, optogenetic inhibition had little effect on the behavioral performance of well-trained animals, but prevented successful reversal learning. We conclude that reactivation of neuronal ensembles in dentate gyrus is necessary to update spatial memories, but is not required for successful navigation in a familiar environment.

Disclosures: P.J. Lamothe-Molina: None. **A. Franzelin:** None. **L.M. Auksutat:** None. **F. Morellini:** None. **T.G. Oertner:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.13/Z33

Topic: H.01. Animal Cognition and Behavior

Support: 80NSSC17K0060 (NASA; AJE)
CHOP Department of Anesthesiology and Critical Care Development Funds (AJE)
PENN McCabe award (SY)

Title: Female C57BL/6J mice exposed to whole-body ^{56}Fe space radiation at astronaut-relevant age show improved touchscreen performance of pattern separation and extinction learning relative to control mice

Authors: *I. SOLER[1,2], F. H. TRAN[2], R. P. REYNOLDS[2], M. J. DESALLE[2], S. YUN[1,2], A. J. EISCH[1,2];
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Abstract: Astronauts during interplanetary space missions will be exposed to galactic-cosmic-radiation, including charged particles like ^{56}Fe. Many studies with mature, “astronaut-aged” rodents suggest space radiation diminishes behavioral performance reliant on dentate gyrus (DG) or prefrontal cortex (PFC) circuits. Given the prevalence of touchscreen testing in astronaut training and the ability of rodent touchscreen tasks to assess the functional integrity of brain circuits in a translationally-relevant way, it is notable that the effect of space radiation on rodent touchscreen performance remains unexplored. Given that prior publications typically show that space irradiation of rats or mice decreases performance in a range of classic behavioral tasks, we initially hypothesized that whole-body ^{56}Fe radiation in mature mice would decrease performance on DG- and PFC-mediated behaviors. However, unpublished work from our lab shows that ^{56}Fe radiation unexpectedly improves performance in a shock-based pattern

separation task in C57BL/6J males. Here we tested whether C57BL/6J female mice exposed to ^{56}Fe radiation (fractionated 20cGy; 600 MeV/n, LET 174 KeV/u) at 6 months of age show improved pattern separation via location discrimination (LD), a reward-based DG-mediated touchscreen task. There was no effect of ^{56}Fe radiation on general touchscreen training or LD-specific training. However, during LD probe testing irradiated female mice discriminated patterns more accurately relative to controls. The same cohorts of mice were next assessed for their ability to learn a new stimulus-response behavior (touching lit square for a reward). While irradiated and control mice learned this new task in a similar number of days, irradiated female mice extinguished this acquired behavior in fewer days relative to controls. Finally, we allowed the same cohorts of mice to undergo a series of behavioral assays probing anxiety-, depression-, and autism-like behaviors. However, irradiated and control mice did not differ in their performance of these behavior tests. Together, these results suggest that ^{56}Fe irradiation has a potentially beneficial effect on pattern separation and extinction learning. Thus, our study is advancing our understanding of the effects of space radiation on mission critical cognitive functions.

Disclosures: I. Soler: None. **A.J. Eisch:** None. **S. Yun:** None. **F.H. Tran:** None. **R.P. Reynolds:** None. **M.J. Desalle:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.14/Z34

Topic: H.01. Animal Cognition and Behavior

Support: R37 NS047344

Title: The role of adult-born granule cells in memory consolidation

Authors: *S. J. TEMME[1], R. PAK[2], G.-L. MING[3], H. SONG[5], K. M. CHRISTIAN[4]; [1]Neurosci., [3]Dept Neurosci, [4]Dept. of Neurosci., [2]Univ. of Pennsylvania, Philadelphia, PA; [5]Neurosci., U Penn, Philadelphia, PA

Abstract: Within the hippocampus, the dentate gyrus has been implicated in complex forms of cognitive processing, including the consolidation and recall of memories. The dentate gyrus is also the site of adult neurogenesis in which new neurons are incorporated into the hippocampal circuit in a dynamic, age-dependent manner. *In vitro* recordings of neurons in the dentate gyrus suggest that immature adult born neurons demonstrate increased excitability and have a lower threshold for plasticity compared to developmentally born cells. This increase in excitability and plasticity suggests that adult born neurons in the dentate gyrus may play a unique role in dentate gyrus functions, including its contribution to learning and memory. While ablation of adult-born

cells has been found to produce deficits in some hippocampal-dependent memory tasks, it is unclear whether these behavioral effects are due to the absence of these cells during consolidation, or a different stage of memory formation and retrieval. Through the inducible expression of ArchaedopsinT or DREADD receptors in adult born cells soon after cell birth, using tamoxifen-mediated Tbr2-Cre, we can selectively inactivate adult born cells at different maturational time points during specific stages of memory encoding in order to examine the influence of these cells on hippocampal neural circuitry and hippocampal-dependent behaviors. Initial studies suggest that inactivation of young adult-born cells for 3-4 hours immediately after training impairs the formation of some forms of hippocampal-dependent memory while sparing others. For instance, inactivation of newly maturing adult born cells in a novel object recognition task is sufficient to impair memory of object location, but not object identity. In contrast, similar inactivation of maturing adult born granule cells immediately after fear conditioning does not appear to affect the expression of fear behavior in these mice in the training context 24 hours later, as compared to their wild-type littermates. These data suggest that young adult-born cells in the dentate gyrus may be involved in the consolidation of some forms of spatial memory without impairing hippocampal dependent memories as a whole.

Disclosures: S.J. Temme: None. **R. Pak:** None. **G. Ming:** None. **H. Song:** None. **K.M. Christian:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.15/Z35

Topic: H.01. Animal Cognition and Behavior

Support: NIH/NINDS (R01 NS111021), "Sex differences in neurotrophin mediated neonatal neuroprotection: Role of ER alpha" (2019-2024). Cengiz, PI
NIH/NINDS (K08 NS088563), “Estrogen receptors and TrkB mediated neuroprotection in neonatal hypoxiaischemia” (2015-2020). Cengiz, PI
NIH/NCATS (UL1 TR002373), “Role of ERα following brain injury: ligand dependent or independent?” (2018-2019). Cengiz, Pilot Grant PI

Title: ERa dependent TrkB mediated novelty seeking behavior is female biased

Authors: B. OZAYDIN[1], D. ZAFER[1], V. CHANANA[1], M. B. HACKETT[1], D. HANALIOGLU[1], P. KEMANLI[1], N. AYCAN[1], N. DEVECI[1], P. FERRAZZANO[2], J. E. LEVINE[3], *P. CENGIZ[2];
[1]Waisman Center, Univ. of Wisconsin, Madison, WI; [2]Dept. of Pediatrics and Waisman Center, Univ. of Wisconsin, Madison, WI; [3]Wisconsin Natl. Primate Res. Ctr., Madison, WI

Abstract: Objective: Neonatal hypoxia-ischemia (HI) related encephalopathy is one of the major causes of learning disabilities and memory deficits in children. Male neonatal brains are more vulnerable to the effects of HI. We reported previously that HI and tyrosine kinase B receptor (TrkB) agonist, 7,8-Dihydroxyflavone (7,8-DHF) administration amplified TrkB phosphorylation and decreased hippocampal apoptosis only in female mice in an estrogen receptor alpha (ERα) dependent way. Here, we investigated the role of ERα in TrkB mediated, female-biased neuroprotection by testing the mice with novel object recognition (NOR) and location (NOL) tests. We hypothesized that TrkB-mediated, female-biased long-term memory and learning is ERα dependent. **Methods:** HI was induced in P9 B6/C57 male and female ERα WT and KO mice by Vannucci's HI model. Mice were treated either with 7,8-DHF or vehicle control, post-HI for 7 days. Recognition and object location memories were assessed by NOR and NOL tests in ERα WT and KO mice at P60+ post-HI. Discrimination ratios (DRs) (time spent with novel object divided by total time spent with both objects) were calculated for 5 min of testing. Mice that have intact novelty-seeking behavior were expected to have DRs above 0.5. ANOVA was used for statistical analysis (mean ± SEM). **Results:** HI decreased the DRs below to 0.5 level for both NOR and NOL tests in ERα WT male (% 28 ± 2 and %23 ± 3) and female (% 28 ± 9 and % 28 ± 4) mice compared to sham WT male (% 72 ± 5 and % 55 ± 6) and female (% 68 ± 4 and % 71 ± 12) mice ($p < 0.001$), respectively. Preferance of familiar object by the mice post-HI suggested that the HI resulted in novelty avoidance behavior. This HI-induced novelty avoidance seen in NOR/L tests were recovered by 7,8-DHF therapy, only in ERα WT females in an ERα-dependent way [% 64 ± 6 and % 67 ± 4, ($p < 0.001$)]. **Conclusion:** 7,8-DHF recovered the novelty-seeking behavior only in female mice in an ERα-dependent way. This might be due to the effect of 7,8-DHF on anxiety-related behavior. We are conducting open field and elevated plus maze tests to determine whether this ERα-dependent female biased neuroprotective mechanism in novelty-seeking behavior is due to decreased anxiety-related behavior or not.

Disclosures: B. Ozaydin: None. **D. Zafer:** A. Employment/Salary (full or part-time):; Waisman Center University of Wisconsin. **V. Chanana:** None. **M.B. Hackett:** None. **D. Hanalioglu:** None. **P. Kemanli:** A. Employment/Salary (full or part-time):; Waisman Center University of Wisconsin. **N. Aycan:** None. **N. Deveci:** None. **P. Ferrazzano:** None. **J.E. Levine:** None. **P. Cengiz:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.16/Z36

Topic: H.01. Animal Cognition and Behavior

Support: KIST 2E29180

Title: Dentate granule and mossy cells exhibit distinct spatiotemporal responses to local change in a one-dimensional landscape of visual-tactile cues

Authors: *D. JUNG[1,2], S. KIM[1,3], A. SARIEV[1], D. KIM[2], S. ROYER[1];
[1]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [3]Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: The dentate gyrus (DG) is critical for detecting changes in environments, however it is unclear how granule cells (GC) and mossy cells (MC), the two excitatory cell types of DG, respond to small changes in the object layout. Here, we record GCs and MCs, identified by spike feature and optogenetic tagging, as mice run on a treadmill belt enriched with visual-tactile cues. We observe that fixing a new cue on the belt induces a reconfiguration of GC and MC spatialrepresentations via the emergence, extinction and rate alteration of firing fields. For both GCs and MCs, the response is maximal near the cue and spread over the entire belt. However, MC response is stronger and more immediate, peaks at a slightly earlier belt position, and exhibits a transient component reminiscent of neuromodulatory activity. We show that a competitive neural network model reproduce GC response contingent to both the introduction of new object-vector inputs and the reconfiguration of MC activity, the former being critical for spreading GC response in locations distant from the cue. These findings suggest that GCs operate as a competitive network and that MCs precede GCs in detecting changes and help expand the range of GC pattern separation.

Disclosures: D. Jung: None. **S. Kim:** None. **A. Sariev:** None. **D. Kim:** None. **S. Royer:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.17/Z37

Topic: H.01. Animal Cognition and Behavior

Title: The role of the neural circuit formation factor LOTUS in cognitive function

Authors: *R. NISHIDA[1], R. ISHIKAWA[2], S. KIDA[2], K. TAKEI[1];
[1]Yokohama City Univ. Grad Sch. of Med. Life Sci., Yokohama, Japan; [2]Tokyo Univ. of Agr., Tokyo, Japan

Abstract: How to overcome the higher brain dysfunction that causes memory disorders, such as dementia, is an issue undergoing research worldwide. The binding of Nogo to Nogo receptor 1 (NgR1) inhibits axonal growth and synaptogenesis; as a result, Nogo is associated with limited neuronal regeneration and impaired memory function. We previously identified lateral olfactory tract usher substance (LOTUS) as an endogenous NgR1 antagonist and suggested a functional

relationship between decreased LOTUS expression in the hippocampus and impaired memory function. However, the physiological role of LOTUS in cognitive function remains unclear. In the present study, we first examined the role of LOTUS in memory function by comparing the performances of wild type (WT), LOTUS gene knockout (LOTUS-KO), and LOTUS gene-overexpressing transgenic (LOTUS-Tg) mice in the social recognition and Morris water maze tests. We found that memory function was impaired in LOTUS-KO mice but was enhanced in LOTUS-Tg mice compared with that in WT mice. Next, we examined the roles of LOTUS in synaptogenesis in cultured hippocampal neurons. We found a decrease in synaptic density in LOTUS-KO mice but an increase in the density in LOTUS-Tg mice. Furthermore, decreased neurogenesis was observed in the hippocampus of adult LOTUS-KO mice, whereas increased hippocampal neurogenesis was observed in adult LOTUS-Tg mice. Because neurogenesis in the hippocampus is known to enhance memory function, increased LOTUS expression could enhance memory function via increased neurogenesis. Notably, LOTUS expression in the hippocampus was shown to gradually decrease with age similar to memory function, which also decreases with age. However, aged LOTUS-Tg mice did not show impaired memory function. These findings suggest that LOTUS is involved in the formation and maintenance of memory as well as synaptogenesis and neurogenesis in the hippocampus.

Disclosures: **R. Nishida:** None. **R. Ishikawa:** None. **S. Kida:** None. **K. Takei:** None.

Poster

086. Hippocampus: Dentate Gyrus

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 086.18/Z38

Topic: H.01. Animal Cognition and Behavior

Support: KIST 2E29180

Title: Distinct impact of spatial and non spatial context manipulations in dentate gyrus

Authors: ***A. SARIEV**[1,2], D. JUNG[1,3], S. ROYER[1];
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Abstract: The Dentate Gyrus (DG) is known to be important for detecting changes in the spatial context, presumably via a pattern separation operation, and receives converging spatial and non-spatial information from medial and lateral entorhinal cortexes, respectively. However, the respective impact of spatial and non-spatial changes on DG representations is largely unknown. Here, we test separately the influence of spatial and non-spatial manipulations of an object layout on DG neurons. We record DG neuron activity using a 64-channels silicone probe while mice

run on a 200-cm-long treadmill belt on which several pairs of small objects are fixed. During a first session, the position of one object was shifted by 12-cm following 20 trials (complete belt rotations). In a second session, the same object was replaced by one of the other objects without any position alteration. We observe that manipulating the object position leads to an equivalent shift of place fields in DG neurons closely associated with the object, such that the spatial extent of DG remapping is relatively restricted to the vicinity of the object. In contrast, replacing the object induces a modification of place field activity that extends throughout the belt. These results indicate that spatial and non-spatial information contribute differently to the profile of DG remapping.

Disclosures: **A. Sariev:** None. **D. Jung:** None. **S. Royer:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.01/Z39

Topic: H.03. Schizophrenia

Title: Deficits in inhibitory control and cortical synchronization in a 15q13.3 microdeletion mouse model

Authors: *M. ZONOUZI, N. SCHUELERT, S. JAEGER, C. DORNER-CIOSSEK, H. ROSENBROCK, V. MACK;
CNS Dis. Res., Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

Abstract: Schizophrenia is a complex neurodevelopmental disorder. Despite intensive research, little progress has been made in developing effective treatments for restoring emotional processing and cognitive function. As a firm link to the underlying network changes, postmortem analysis consistently indicated deficits in cortical parvalbumin-expressing (PV+) interneurons and the surrounding perineuronal nets (PNNs). Amongst the variety of animal models that replicate cortical network deficits, mouse models for rare and schizophrenia-linked copy number variants (CNVs) provide a unique opportunity to link strong construct validity with pathological findings and relevant biomarkers. Mouse models for the highly penetrant 15q13.3 microdeletion have been shown to mimic the decrease in PV+ cell density and abundance of PNNs in the prefrontal cortex. Cellular deficits were accompanied by changes in evoked electroencephalography (EEG) patterns such as a reduced response at gamma frequency, matching with markers of impaired network function in patients with schizophrenia. Using a combination of high-content histology, slice electrophysiology and EEG, we examined cortical excitatory-inhibitory (E/I) balance and network activity in adult Df(15q13)-/+ mice. We could show a significant reduction in power and coherence of the 40Hz auditory steady state response (ASSR) in the auditory and prefrontal cortex of CNV mice, indicating impaired cortical network

synchronization in the high frequency range. Both ASSR deficits have been restored by pharmacological modulation of glutamatergic transmission. These findings provide evidence for the contribution of increased excitation to cortical E/I imbalance and imprecise sensory processing in the Df(15q13)/+ mouse model, which might mimic schizophrenia risk and aspects of the disease pathology.

Disclosures: **M. Zonouzi:** None. **N. Schuelert:** None. **C. Dorner-Ciossek:** None. **H. Rosenbrock:** None. **V. Mack:** None. **S. Jaeger:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.02/Z40

Topic: H.03. Schizophrenia

Support: College of Medical and Veterinary Life Science Doctoral Training Programme - University of Glasgow

Title: Brain-derived neurotrophic factor and JNK-signalling modulate perineuronal net development and maturation of cultured cortical interneurons: Implications for schizophrenia-related 16p11.2 duplication syndrome

Authors: *A. WILLIS[1], J. A. PRATT[2], B. J. MORRIS[1];
[1]Inst. of Neurosci. and Psychology, Univ. of Glasgow, Glasgow, United Kingdom; [2]Inst. of Pharm. and Biomed. Sci., Univ. of Strathclyde, Glasgow, United Kingdom

Abstract: Schizophrenia risk arises from interactions of multiple common genetic variants, or, in rare cases, single variants (such as duplications of chr. 16p11.2) carry a more powerful genetic risk. Gene-environment interactions which impact CNS development further complicate schizophrenia aetiology.
Cortical GABAergic interneurons are robustly dysfunctional in schizophrenia. Reductions in glutamic acid decarboxylase (GAD65/67) and deficits in extracellular matrix structures, perineuronal nets (PNNs), which enwrap parvalbumin-containing interneurons have been observed in patients.
Given their complex developmental pattern, these interneurons may be particularly vulnerable to genetic and environmental perturbations. Here, we investigate genetic risk factors affecting JNK signalling (a family of mitogen activated protein kinases intrinsic to cortical interneuron development), along with brain-derived neurotrophic factor (BDNF), a GABAergic interneuron modulator which is reduced in post-mortem CNS tissue from schizophrenia patients.
Wild-type (WT) mouse primary cortical neuronal cultures were treated at 7 days *in vitro* (DIV) with vehicle, BDNF (50ng/ml), JNK-inhibitor (SP600125) or BDNF+JNK-inhibitor for 7 DIV.

BDNF-TrkB activated signalling pathways were inhibited: ERK (PD98059) and PI3K (wortmannin). Subsequently, primary cortical neurons from mice with a mutation equivalent to the human 16p11.2 duplication (DUP mice) or paired WTs were treated at 7 DIV with either vehicle or BDNF for 7 DIV (N=18 per condition).
PNN development was assessed using signal intensity of *Wisteria floribunda agglutinin* (WFA) lectin, which labels PNNs. Immunoblotting was performed for GAD65/67 and phosphorylated JNK (pJNK).
In WT cultures, BDNF significantly increased WFA intensity; an effect which was diminished when JNK signalling was inhibited. WFA intensity was also reduced with JNK inhibition alone. BDNF significantly increased GAD67 levels compared to vehicle, and this effect was negated with JNK inhibition, but not with ERK or PI3K inhibition.
Compared to WT controls, DUP neurons developed abnormally in culture. WFA intensity in DUP cultures was higher than in WTs, suggesting increased PNN density. pJNK in DUP neurons was elevated compared to WTs. BDNF increased GAD67 compared to vehicle in both genotypes. Interestingly, pJNK levels were increased after BDNF treatment only in WT cultures. BDNF enhanced GAD67 and PNN maturation in developing cortical interneurons; an effect dependent on JNK signalling. Our data indicate a regulatory role of BDNF and JNK in PNN development, and conceivably, with PNN abnormalities in 16p11.2 duplication syndrome.

Disclosures: A. Willis: None. **J.A. Pratt:** None. **B.J. Morris:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.03/Z41

Topic: H.03. Schizophrenia

Title: Co-localization of EQTL and GWAS in schizophrenia

Authors: L. MA[1], S. CHETTY[1,2];
[1]Dept. of Psychiatry and Behavioral Sci., [2]Inst. for Stem Cell Biol. and Regenerative Med., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Schizophrenia is a debilitating psychiatric condition affecting roughly 0.7% of adults. It is highly heritable and polygenic. A recent genome-wide association study identified 145 loci that confer risk for schizophrenia, but the underlying mechanisms remain largely unknown. Our overarching goals in this study are to identify mechanisms that underlie genetic risk by investigating the role of disease related SNPs on regulating gene expression. In this study, we use RNA sequencing and whole genome sequencing data generated from 1,479 human postmortem brain samples across 13 regions including prefrontal cortex. All samples originate from tissue collections of the GTEx consortium. Gene counts were based on GENCODE Release

19 (GRCh37.p13), 56,203 transcripts in total. Genes with average counts < 0 were excluded. A total of 10,411,470 SNPs were retained after filtering SNPs that did not fulfill genotype missing rate >10%, HWE at p-value < 1e-5 and MAF < 0.01. We modeled expression after transforming with log2 with an offset of 1 by liner regression. We performed eQTL analyses using the MatrixEQTL by allowing for a 1MB window around each SNP, and adjusting for ancestry PCs, expression PCs, and sex. SMR and mapping were used for co-localization analysis. To assess gene expression alteration by schizophrenia risk loci, we co-localized the eQTL results and schizophrenia GWAS summary statistics. We identified 55 genes (32 genes located within MHC) by SMR methods and 89 genes (51 genes located within MHC) across the 13 brain regions by mapping using SMR default threshold (5e-8) and filtering schizophrenia GWAS non-significant SNPs (5e-8). It is worth noting that all SMR genes were replicated by independent mapping. DLPFC has been demonstrated to be strongly associated with schizophrenia. A total of 11 genes were identified in this brain region using the two methods. We next took an unbiased data-driven approach and performed a comprehensive gene-set enrichment analysis across the 13 tissues. Interestingly, we determined a KEGG pathway that is involved in neuropsychiatric drug metabolism process (Tamoxifen, Morphine and Codeine, and Citalopram) (FDR=4.78E-10 and fold enrichment=3.74 in prefrontal cortex; FDR range: 7.86E-03 to 1.61E-11; fold enrichment range: 2.35-4.77). In summary, our findings provide new targets for modeling schizophrenia risk at the molecular level.

Disclosures: L. Ma: None. **S. Chetty:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.04/Z42

Topic: H.03. Schizophrenia

Support: NIMH R01MH095995
UTMB Jeane B. Kempner Predoctoral Fellowship
UT System BRAIN Initiative.

Title: Role of the glycogen synthase kinase 3 pathway in the pathophysiology of schizophrenia

Authors: *J. DI RE[1,2], G. BOTELLO-LINS[3], L. STERTZ[4], H. RAVENTOS[5], C. WALSS-BASS[4], F. LAEZZA[2];
[1]Neurosci. Grad. Program, [2]Dept. of Pharmacol. and Toxicology, Univ. of Texas Med. Br., Galveston, TX; [3]Clear Falls High Sch. Biotech. Practicum, Clear Falls ISD, Texas City, TX; [4]Dept. of Psychiatry and Behavioral Sci., Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [5]Dept. of Cell. and Mol. Biol., Univ. of Costa Rica, San Jose, Costa Rica

Abstract: The biological mechanisms underlying schizophrenia (SZ) are still not well understood. Studies and clinical evidence suggest that multiple environmental and genomic risk factors contribute to the risk of developing SZ. Integrative translational approaches that include *in vitro* models and fine-tuned human genetic studies are necessary to elucidate the contribution of these genomic risk factors to endophenotypes of SZ. Emerging evidence indicates that dysregulation of the protein kinase B (Akt)/glycogen synthase kinase 3β (GSK3β) pathway is a risk factor for SZ. As such, there is a need to understand the molecular targets of this signaling pathway. We have previously shown that GSK3β regulates the complex assembly and protein:protein interactions (PPI) within the voltage-gated Na+ (Nav) channel complex at the axon initial segment (AIS), the action potential initiation site. The AIS contains a nexus of scaffolding and regulatory proteins, which ensure the proper targeting, and function of Nav channels, including Neurofascin (NFASC), Fibroblast Growth Factor 14 (FGF14) and β-IV Spectrin (SPEC). Based on this premise we hypothesized that dysfunction of the GSK3/Akt pathway could disrupt PPI at the AIS contributing to molecular endophenotypes of SZ. By integrating genomic and immunocytochemical studies in neurons differentiated from induced pluripotent stem cells (iPSCs) from a small, homogeneous population with SZ we have found a decrease in the mRNA level of GSK3β in SZ patients ($p<0.05$, $n=11$, T-test with Welch's Corrections) compared to controls. Using confocal microscopy, we have found that patients have a decreased average level of NFASC compared to controls ($p<0.05$, $n=22$, Student's T-test), and treatment with an inhibitor of Wee1, a kinase that regulates ubiquitination of GSK3, reverses this phenotype, leading to an increase in average level NFASC in patients compared to controls ($p<0.5$, $n=20$, Student's T-test). No significant changes were seen in the average level of FGF14 between patients and controls, but treatment with an AKT inhibitor decreases the average level of FGF14 in patients, but not in controls. Patients also had lower average levels of SPEC ($p<0.01$, T-test with Welch's corrections), but no inhibitor treatments affected the average level of SPEC at the AIS in patients or controls. Overall, these results indicate that neurons from patients with SZ are sensitive to perturbations of the GSK3/Akt pathway that affect the AIS composition, exhibiting unique phenotypes not seen in neurons from healthy controls.
This research was funded by NIMH R01MH095995, UTMB Jeane B. Kempner Predoctoral Fellowship, UT System BRAIN Initiative.

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Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.05/AA1

Topic: H.03. Schizophrenia

Title: Evaluation of the cell type-specific expression of schizophrenia risk-related transcripts in postmortem human dorsolateral prefrontal cortex using single molecule *in situ* hybridization

Authors: *Y. TAKAHASHI[1,2], K. R. MAYNARD[1], M. TIPPANI[1], A. E. JAFFE[1,3,4,5,6], K. MARTINOWICH[1,6,7], D. R. WEINBERGER[1,5,6,7,8], T. M. HYDE[1,6,8];
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Abstract: Genome-wide association studies have now identified many single nucleotide polymorphisms (SNPs) associated with risk for schizophrenia (SCZ). As most risk-associated SNPs fall in non-coding regions, they are hypothesized to influence risk by affecting expression levels and/or splicing of transcripts. In previously published studies, we have integrated genomic risk data with bulk RNA-sequencing from postmortem human brains of SCZ cases and controls to identify genes and specific transcript variant classes associated with increased risk for SCZ. In particular, we identified exon 2- and 3-deleted variants of arsenic methyltransferase (*AS3MT*) and the exon 9-deleted variant class of sorting nexin-19 (*SNX19*) as SCZ risk transcripts. However, given that RNA-seq data were derived from bulk homogenized tissue, detailed anatomic localization of these risk transcripts remains unclear. Obtaining cortical laminar and cell type-specific expression data can increase our understanding of how these molecular associations functionally contribute to risk. Here, to elucidate the localization of *SNX19* and *AS3MT* transcripts in specific cortical layers and cell types in postmortem human dorsolateral prefrontal cortex (DLPFC), we employed RNAscope multiplex single molecule *in situ* hybridization (ISH) technology. We analyzed *SNX19* and *AS3MT* expression in neurons, oligodendrocytes, and astrocytes in 10 postmortem brains of neurotypical control cases aged 17- to 27-years with risk or non-risk genotypes. Quantification of single fluorescent molecules from target probes as well as co-localization with cell type-markers was performed by a novel computational program, which we developed for accurate and reproducible analysis to reduce the effects of lipofuscin autofluorescence. Cell type-specific expression was further compared by genotype to illustrate the potential influence of risk polymorphisms on transcript regulation. Finally, we utilized cutting-edge ISH technology, BASEscope, which can detect transcript variants differing by a single exon junction, to delineate the localization of risk-associated splice variants in specific cell types. Our results showed that *AS3MT* and *SNX19* transcripts were more preferentially expressed in neurons than in non-neurons. Furthermore, we determined that *SNX19* expression was similar in GABAergic and glutamatergic neurons, while *AS3MT* transcripts were more preferentially expressed in GABAergic neurons. These findings suggest that individual SCZ risk-transcripts have distinct localization patterns in the healthy human brain and that perturbations of these patterns could contribute to pathogenesis in SCZ.

Disclosures: **Y. Takahashi:** None. **K.R. Maynard:** None. **M. Tippani:** None. **A.E. Jaffe:** None. **K. Martinowich:** None. **D.R. Weinberger:** None. **T.M. Hyde:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.06/AA2

Topic: H.03. Schizophrenia

Support: SUHF G01170502
Sovargen G01190133

Title: Identification of low-level brain somatic mutations in dorsolateral prefrontal cortex underlying schizophrenia

Authors: ***M.-H. KIM**[1], J. LEE[2], J. PARK[1], I. KIM[1], K. KIM[1], S. PARK[1], S. KIM[1], Y. AN[1], S. KIM[3], J. LEE[1];
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Abstract: Genetic factors are considered as important for etiology of schizophrenia (SCZ). However, most cases of SCZ are known as sporadic. Many researchers have tried to find the genetic cause of SCZ using DNA extracted from the patients' blood. As a result, some related genes and copy number variations were found. However, only up to 5% of cases can be explained by these variations.
One major finding in the pathology of SCZ is the hypofrontality which indicates reduced metabolic activity in prefrontal cortex, especially in DLPFC. Moreover, recent study shows strong correlation between SCZ disease gene expression and DLPFC. This fact lead us to suspect that somatic mutations in DLPFC might cause the SCZ symptoms.
First of all, we performed 500x deep whole exome sequencing on matched DLPFC and peripheral tissues of 26 healthy controls and 27 SCZ patients which are provided by Stanley Medical Research Institute. We found average 4.9 and 6.0 in DLPFC and 8.6 and 7.3 mutations in peripheral tissues of SCZ and controls. The pattern of somatic mutations was not significantly different in SCZ and controls. This result means randomly developed mutations in important genes in neurons can induce psychiatric disease. Surprisingly, ingenuity pathway analysis shows that these mutated genes detected only in DLPFC of SCZ are enriched on canonical pathways which are known as disrupted in SCZ, related to glutamate and dopamine receptor signaling.
To examine the functional role of deleterious somatic mutations in the DLPFC of SCZ, we tested missense mutations in *GRIN2B* which detected in two cases. *GRIN2B* is very well-known genes which is related to SCZ. We found that both mutations lead disruption of GRIN2B localization but not its own expression pattern through primary culture experiment. This may affect patients'

DLPFC activity and cause malfunction of synaptic plasticity and memory function and this should be studied more to figure out which neuronal connection has affected. Overall, our study suggests new insight into genetic architecture and molecular genetic cause of psychiatric disease. Studies to find out the factors which break neural activity may induce severe symptoms of SCZ need to be proceed to understand the causes of SCZ symptoms and develop infallible cure.

Disclosures: **M. Kim:** None. **J. Lee:** None. **J. Park:** None. **I. Kim:** None. **K. Kim:** None. **S. Park:** None. **S. Kim:** None. **Y. An:** None. **S. Kim:** None. **J. Lee:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.07/AA3

Topic: H.03. Schizophrenia

Support: KAKENHI

Title: Lack of accumbal trace amine neurons in schizophrenia: Key of disease progression

Authors: *K. IKEMOTO;
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Abstract: Due to complexity of the human central nervous system (CNS), there is an urgent necessity to elucidate pathophysiology of disorders in the human CNS. I specified anatomical subgroups of human CNS D-neurons (dopa decarboxylating neurons = trace amine (TA) neurons, type 1) (Ikemoto 2016), demonstrated D-neuron decrease in the nucleus accumbens (Acc, D16) of postmortem brains with schizophrenia (Ikemoto et al. 2003), and established “D-cell hypothesis (TA hypothesis) of schizophrenia” (Ikemoto 2012). The human D-neuron system is far developed in the forebrain in comparison with that of other species, including non-human primates (Kitahama et al. 2009). The TAAR1 (TA-associated receptor 1), exclusive receptor of TAs in humans, has a large number of ligands including tyramine, β-phenylethylamine and methamphetamine, which affect on human mental states. The "D-cell hypothesis" is that accumbal D-neuron decrease in schizophrenia and consequent TAAR1 stimulation decrease to terminals of midbrain ventral tegmental area (VTA) DA neurons induces mesolimbic DA hyperactivity (Bradaia et al. 2009) of schizophrenia. Dysfunction of subventricular neural stem cells (NSC) located in Acc (Sanai et al. 2004) is the cause of D-neuron decrease in Acc (Ikemoto 2012). “D-cell hypothesis”, linking DA dysfunction hypothesis to NSC dysfunction hypothesis, explains mechanisms of mesolimbic DA hyperactivity and disease progression of schizophrenia, and predicts effectiveness of TAAR1 agonists or TAAR1 partial agonists (Revel et al. 2013) and neurotropic substances (e.g., brain-derived neurotrophic factor (BDNF), lithium, anticonvulsants, and antidepressants). In future, mesenchymal stem cell (MSC)-induced D-neurons would be

available for regenerative medicine of neuropsychiatric disorders, including degenerative disorders such as dementia, as well as developmental disorders. D-neuron-TAAR1 signals in pathophysiology of neuropsychiatric disorders should further be explored.

Disclosures: K. Ikemoto: None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.08/AA4

Topic: H.03. Schizophrenia

Support: NIMH-IRP

Title: Micro-RNA 137 host gene transcripts in schizophrenia: A postmortem study

Authors: N. FENG[1], A. MANDAL[1], N. AKULA[2], R. KRAMER[1], B. KOLACHANA[1], Q. XU[1], F. J. MCMAHON[2], P. AULUCK[1], *S. MARENCO[1], B. K. LIPSKA[1];
[1]NIMH/HBCC, Bethesda, MD; [2]NIMH/Human Genet. Br., Bethesda, MD

Abstract: Background: Although growing genetic and biological evidence suggests an important role of MIR137 in schizophrenia and other psychiatric disorders, the mechanistic causes are still unknown. To elucidate the genetic association between schizophrenia risk single nucleotide polymorphisms (SNPs) in the MIR137 locus, we measured differential expression of miR-137 in schizophrenia and genetic effects of the two strongest risk SNPs rs1625579 and rs1702294 in the dorsolateral prefrontal cortex (DLPFC) of a large cohort of postmortem cases with and without serious mental illness, but no significant effects were found (data not published). Since the host gene of MIR137 (MIR137HG) may play a regulatory role as a long non-coding RNA (lncRNA), we have characterized expression of its major transcripts and their association to genetic variants in large cohorts of postmortem brain samples from the subgenual anterior cingulate cortex (sgACC) and DLPFC.
Subjects & Methods: RNA expression was measured with RNA-Seq and qPCR in the sgACC (Controls: 54; Bipolar: 33; MDD: 51; SZ: 43) and DLPFC (Controls: 310; Bipolar: 54; MDD: 130; SZ: 164). Genotyping was done using Illumina BeadArray chips and imputation was done via the Sanger Imputation Server.
Results: A novel transcript containing 6 distal exons of MIR137HG was found by the RNA-Seq in the sgACC and DLPFC and confirmed by PCR based approaches. We found that the expression of two major MIR137HG transcripts in sgACC were much higher than they were in DLPFC by qPCR (20 samples for each region; T-test $p < 1 \times 10^{-6}$). In the sgACC, no differential expression was found in any of the three psychiatric disorders compared to non-psychiatric controls by either qPCR or RNA-Seq. More than 10 independent schizophrenia risk SNPs were

examined for genetic effects on MIR137HG expression in the sgACC, but none of them showed a significant effect after multiple test correction. In the sgACC RNA-seq analysis, no expression quantitative trait locus was found affecting MIR137HG transcripts.
Conclusion: MIR137HG lncRNA transcripts don't appear to be associated with schizophrenia and schizophrenia risk SNPs in the sgACC, where MIR137HG is highly expressed. DLPFC data on MIR137 HG transcripts from qPCR and RNA-seq have been acquired also, and will be analyzed.

Disclosures: **N. Feng:** None. **A. Mandal:** None. **N. Akula:** None. **R. Kramer:** None. **B. Kolachana:** None. **Q. Xu:** None. **F.J. McMahon:** None. **P. Auluck:** None. **S. Marenco:** None. **B.K. Lipska:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

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Program #/Poster #: 087.09/AA5

Topic: H.03. Schizophrenia

Support: LB692 to Dr. Holly Stessman
Haddix faculty research fund to Dr. Annemarie Shibata

Title: Schizophrenic psychosis symptoms on a background of mild to moderate carnitine palmitoyltransferase II deficiency: A case report

Authors: *R. N. WICKRAMASEKARA[1], J. NGO[2], S. ANDERSON[3], D. WILTON[3], V. KOLLI[5], A. SHIBATA[4], H. STESSMAN[1];
[1]Dept. of Pharmacol., [2]Dept. of Psychiatry, [4]Dept. of Biol., [3]Creighton Univ., Omaha, NE; [5]CHI Hlth., Omaha, NE

Abstract: Schizophrenia is a multifaceted mental illness characterized by cognitive and neurobehavioral abnormalities, psychopathology and a complex genetic etiology. Carnitine palmitoyltransferase II (CPT II) deficiency is a metabolic disorder resulting in impaired transport of long-chain fatty acids from the cytosolic compartment to the mitochondrial inner membrane where fatty acid β oxidation takes place. Reports suggest that subjects with schizophrenia have a higher incidence of metabolic and bioenergetic dysfunction. Here we present an interesting clinical case of an adolescent male that presented with schizophrenic psychosis with a history of mild to moderate CPT II deficiency. At present it is not clear whether the metabolic deficiency that was prominent in early childhood was causal in the later onset neuropsychiatric manifestation. Therefore, this study is aimed at identifying potentially pathogenic; inherited or de novo genetic variants in the proband that maybe causal in the observed psychiatric phenotypes. Notable neonatal clinical presentations included two hypoglycemia-induced seizures, a history of

lethargy and fatigue. Metabolic testing for recurrent hypoglycemia showed elevated acyl carnitine levels. CPT II activity levels measured in cultured patient fibroblasts showed a ~50% reduction compared to controls consistent with CPT II haploinsufficiency. Therefore, the patient was started on carnitine supplementation. Symptoms were further managed using a low-fat, complex carbohydrate diet. At the age of 7, the proband was diagnosed with ADHD and at the age 17, first presented with psychosis demonstrating persecutory ideation, ideas of reference, thought disorder/blocking, paranoia, delusions, word salad and nonsensical conversation. An MRI scan showed encephalomalacia and paramedian occipital lobes. Vyvanse and a reduced dose of risperidone was followed by a psychotic exacerbation.
To identify genetic variants that may be causal in the observed patient phenotypes, whole-exome sequencing was performed using salivary DNA from the proband, unaffected twin sister and both biological parents. Variant calling performed on samples showed the proband carried a homozygous p.VAL368ILE mutation and a heterozygous p.MET647VAL mutation in CPT II, which together may explain the reduced enzymatic efficiency. Notable variants were also identified in apolipoprotein E and a precursor of dopamine-β-hydroxylase, all of which were validated by Sanger sequencing.
Ongoing analysis of the sequencing data will attempt to elucidate the effect(s) of variants related to the phenotypes observed.

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Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

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Topic: H.03. Schizophrenia

Support: Joseph P. Healey Award
University of Massachusetts Medical School

Title: Diminished Ca^{2+}signaling, Cav1 Ca^{2+} channel expression and activity in pancreatic beta cells expressing truncated DISC1 (Disrupted in Schizophrenia 1)

Authors: P. LU[1], R. SHARMA[2], L. ALONSO[2], R. ZHUGE[1], A. JURCZYK[2], ***A. R. RITTENHOUSE**[3];
[1]Microbiology & Physiological Systems, [2]Diabetes Ctr. of Excellence, Univ. of Mass Med. Sch., Worcester, MA; [3]Microbiology & Physiological Systems, Univ. Massachusetts Med. Sch., Worcester, MA

Abstract: In addition to cognitive impairment, schizophrenics often suffer from non-obese Type 2 Diabetes (T2D). Here, we examined whether similar cellular changes occur in pancreatic β-cells as found in neurons from a genetic model of schizophrenia. Specifically, we tested whether β-cells exhibit disrupted excitation-secretion coupling when a truncated human DISC1 (*th*DISC1) gene, originally discovered in a Scottish family having high penetrance for major mental illness such as bipolar disorder and schizophrenia, is expressed selectively in mouse pancreatic β-cells upon ingesting doxycycline (DOX+). Expression of *th*DISC1 significantly decreased blood insulin levels and glucose-stimulated insulin secretion (GSIS) from isolated mouse islets, revealing an independent role for DISC1 in β-cells. In central neurons, DISC1 regulates Ca_V2 Ca^{2+}-channel expression, Ca^{2+} influx and transmitter release. To determine whether DISC1 similarly regulates Ca^{2+} physiology in β-cells, we tested *th*DISC1 DOX+ versus DOX- β-cells for changes in: *i*) intracellular Ca^{2+}signaling using Fluo 3-AM; *ii*) Ca_V1 channel expression, the dominant channel class controlling GSIS; *iii*) and Ca_V1 activity using whole-cell recording methods. We found increases in $[Ca^{2+}]_i$ following 20 mM glucose were delayed >2 min and diminished ~50% in dissociated β-cells expressing *th*DISC1. The wild-type profile could be partially rescued when GSK3β was inhibited. When sections of pancreas were stained with an anti-$Ca_V1.2/1.3$ antibody (Antibodies Inc), *th*DISC1 DOX+ vs DOX- insulin-positive cells exhibited decreased staining. Lastly, peak inward Ba^{2+} currents and FPL 64176 induced long-lasting L-type tail currents decreased in *th*DISC1 DOX+ (56% & 67% respectively) vs DOX- β-cells at all step potentials tested. No obvious change in activation kinetics occurred. Human donor β-cells, infected with adeno-sh-DISC1, exhibited similar decreases in currents compared to wild-type cells. These changes in excitation-secretion coupling in β-cells parallel those in neurons expressing *th*DISC1. Thus, at the cellular level, truncated *th*DISC1 precipitates disorders of secretion that associate with schizophrenia and T2D. These findings provide a molecular link for a prevalence of diabetes in individuals with psychiatric disorders.

Disclosures: P. Lu: None. **R. Sharma:** None. **L. Alonso:** None. **R. ZhuGe:** None. **A. Jurczyk:** None. **A.R. Rittenhouse:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

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Program #/Poster #: 087.11/AA7

Topic: H.03. Schizophrenia

Support: R03 MH 104851
R03 AG 052120

Title: Perineuronal net aberrations as a putative mechanism of behavioral and neural alterations in DISC-1 mutation model of schizophrenia

Authors: *R. SULTANA, C. C. LEE;
Comparative Biomed. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Schizophrenia is a disorder characterized by variable etiology leading to behavioral and molecular changes in the affected individuals. Among the various cellular and extracellular changes in this disorder, perineuronal nets (PNNs), an extracellular matrix component (ECM) found closely associated with parvalbumin-positive fast spiking interneurons (PVs) exhibit a major pathology. Functionally, PNNs protect these interneurons from oxidative stress by maintaining the ionic balance in the micro-environment of these metabolically hyperactive cells. These ECM structures are found to be dysregulated in the prefrontal, entorhinal cortex and hippocampus of human schizophrenia subjects. Here in this study, we used a genetic mouse model (with mutation in *DISC1-Disrupted in Schizophrenia*) to study behavioral and neural characteristics of genetically predisposed animals along with their development of PNNs and PVs. When tested behaviorally, we found that *DISC-1* mutation mice exhibit schizophrenia-like phenotype, exhibiting positive (hyperlocomotion, disrupted PPI), negative (decreased mobility in forced swim and tail suspension tests), and cognitive (decreased time in correct arm in y-maze task) changes. Electrophysiologically, spontaneous neuronal firing is significantly reduced in the hippocampus of these animals and that closely associated neurons fire action-potentials that are out-of-phase with reduced burst firing patterns compared to wild-type controls. Most interestingly, we found reduced inter-stimulus interval peaks and an increased hyper-polarization phase during recorded action potentials. Further, molecular studies in these animals determined that an over proliferation of PNN structures were present in the prefrontal cortex, whereas there is a decreased colocation of PNNs and PVs along with significantly reduced number of the PVs. Thus, we conclude that the resultant aberrant firing pattern in the *DISC1* mutation model could be a result of an abnormal expression of PNNs, perhaps due to oxidative damage to PVs or altered neuronal membrane properties.

Disclosures: R. Sultana: None. **C.C. Lee:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

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Program #/Poster #: 087.12/AA8

Topic: H.03. Schizophrenia

Support: Albstein Research Scholarship
NIH Grant P50MH094268

Title: Neural correlates of conditioned hallucinations in a ketamine mouse model of schizophrenia

Authors: J. WU, R. P. HABERMAN, M. GALLAGHER, *M. KOH;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: When two stimuli such as a tone and a visual stimulus are repeatedly presented together, individuals with psychosis tend to report hearing the tone subsequently in response to the visual stimulus alone, which underscores the importance of prior associative experiences in driving hallucinatory perception (Powers et al., 2017). Such conditioned hallucinations involved true percepts as evidenced by increased neural activation in tone-responsive regions during the conditioned hallucinations. Based on the same principle, we have previously shown that mice used to model schizophrenia were more susceptible to conditioned hallucinations as instantiated by a greater tendency to use mental representations of a prior gustatory experience to form associations in learning (Koh et al., 2018). Here, we examined the neural correlate of such a gustatory experience that could form the basis of conditioned hallucinations in a ketamine mouse model. Adolescent mice were exposed to either ketamine or vehicle for two weeks, and trained drug free in adulthood to associate a taste with an odor. The mice were then tested with the odor alone to evoke a conditioned taste hallucination. Our results showed that compared to control mice, ketamine-exposed mice showed a significantly stronger neural activation as measured by c-Fos mRNA expression in the gustatory (taste) cortex to the odor, suggesting that the ketamine-exposed mice experienced a stronger neural perception of the taste representation that was associated with the odor. We next examined the contribution of the hippocampus in this paradigm with the ketamine mouse model previously shown to exhibit hippocampal hyperactivity that drives hyper-responsiveness of the dopamine system. We hypothesized that lesions targeting the hippocampus would eliminate hippocampal hyperactivity and its downstream effects including attenuating hyperdopaminergic function along with the tendency for conditioned hallucinations. Contrary to our expectation, ketamine-exposed mice with hippocampal lesions showed increased locomotor responses to the dopamine agonist amphetamine compared to ketamine-exposed mice with sham lesions. In addition, after repeated odor-taste pairings, the predictive odor evoked a stronger neural taste activation in the mice with hippocampal lesions than control mice, suggesting damage to the hippocampus can lead a greater susceptibility to psychosis-like behavior.

Disclosures: J. Wu: None. **R.P. Haberman:** None. **M. Gallagher:** None. **M. Koh:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.13/AA9

Topic: H.03. Schizophrenia

Support: NIH Grant MH115188

Title: Neural circuits underlying hyperlocomotive behaviors induced by 14-3-3 deficiency in the hippocampus of mice

Authors: *J. ZHANG, Y. WU, Y. ZHOU;
Florida State Univ., Tallahassee, FL

Abstract: Schizophrenia is a debilitating psychiatric disorder characterized by a variety of symptoms. The positive symptoms of schizophrenia may stem from a disruption in higher brain control over the dopaminergic system, yet the key forebrain brain regions and the underlying neurocircuits remain poorly understood. Recent human studies have implicated early involvement of the hippocampus in the pathophysiology of schizophrenia. Our recent studies found that virus-mediated bilateral inhibition of 14-3-3 proteins in the dorsal hippocampus alone can induce hyperlocomotive behaviors in mice. 14-3-3 proteins are a family of homologous proteins highly expressed in the brain. They are genetically linked to several neuropsychiatric disorders, including schizophrenia. Mice with 14-3-3 deficiency in the forebrain exhibit schizophrenia-like behaviors along with an elevated striatal dopamine level. Dysregulation of 14-3-3 in the hippocampus has shown to alter synaptic transmission and plasticity, thus we hypothesize that 14-3-3 inhibition disrupt hippocampal output, which leads to overactivation of the dopaminergic system through a multisynaptic pathway. To test the hypothesis, we utilized anterograde adeno-associated virus (AAV) expressing YFP and retrograde neurotracers (Alexa488-conjugated cholera toxin subunit B) to map the potential anatomical connections between the dorsal hippocampus and the midbrain dopamine system. Furthermore, by using AAV-mediated circuit-specific 14-3-3 inhibition together with chemogenetic circuit manipulations, we expect to identify the functional involvement of these neural circuits underlying the observed hyperlocomotive behaviors in mice.

Disclosures: J. Zhang: None. **Y. Wu:** None. **Y. Zhou:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

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Program #/Poster #: 087.14/AA10

Topic: H.03. Schizophrenia

Title: DGCR2, a schizophrenia risk gene, regulates synaptic transmission, anxiety and hippocampal-prefrontal synchrony

Authors: *H. L. ROBINSON[1], B. LUO[2], W. CHEN[2], Z. TAN[1], P. CHENG[2], M. XIONG[2], E. FEI[2], L. MEI[1];
[1]Case Western Reserve Univ. Dept. of Neurosci., Cleveland, OH; [2]Nanchang Univ. Inst. of Life Sci., JiangXi, China

Abstract: Chromosome 22q11.2 deletion syndrome is due to a common deletion that causes an array of developmental defects including DiGeorge syndrome and velocardiofacial syndrome. One percent of Schizophrenia patients possess Chromosome 22q11.2 microdeletion syndrome, whereas up to 30% of Chromosome 22q11.2 syndrome patients display symptoms diagnosable of schizophrenia. The 22q11.2 region contains 46 genes including *DGCR2* (DiGeorge Syndrome Critical Region Gene 2), which encodes a 550-amino acid putative adhesion protein. Yet, the physiological function of DGCR2 in the brain remains unclear. To address this, we generated *DGCR2* deficient mice *DGCR2-LacZ* (*LacZ*/*LacZ*) where the *DGCR2* gene is replaced by a cassette containing *lacZ.* We found that the β-galactosidase activity was detectable in a number of regions in the mouse brain, with abundant expression in the hippocampus (HPC) and cortex. Immunostaining indicated that *DGCR2* was expressed in pyramidal neurons and was co-localized to PSD-95 puncta at the synapses. *DGCR2* deficient mice exhibited anxiety-like symptoms in the Open Field Test (OFT) and Elevated Plus Maze (EPM), by a decreased time spent in the center and open arms, respectively. This anxiety-like phenotype is intriguing as 40-70% of Chromosome 22q11.2 deletion syndrome and around 40% of Schizophrenia patients suffer from anxiety disorders. Synchronized neural activity between the HPC and prefrontal cortex (PFC) has not only shown to be involved in anxiety-like behavior, but disrupted in mouse models of Chromosome 22q11.2 deletion syndrome and Schizophrenia. To test this, we recorded local field potentials and neuronal spikes in the HPC and PFC during the OFT and EPM. Interestingly, we found a decrease in synchronous activity between the HPC and PFC during OFT and EPM of *DGR2* deficient mice, especially in the anxiogenic portions of the behavior. Our study reveals novel functions of *DGCR2*, specifically in excitatory transmission and HPC-PFC synchrony that might underlie the pathology of anxiety disorders, Chromosome 22q11.2 deletion syndrome and Schizophrenia.

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Poster

087. Schizophrenia: Animal Models and Genetic Studies

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Topic: H.03. Schizophrenia

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Helen S. Vernik Schizophrenia Pilot Research Project Grant, 2017

Title: Targeting a disrupted population of prefrontal parvalbumin interneurons to rescue cognitive deficits in a rat model of schizophrenia

Authors: *L. CHAMBERLIN[1], B. R. FERGUSON[2], E. P. MCEACHERN[1], W. J. GAO[1]; [1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA

Abstract: Schizophrenia (SZ) is a severe psychiatric illness characterized by positive, negative, and cognitive symptoms. While cognitive deficits are most predictive of functional outcome, these symptoms remain the least responsive to existing treatments. Postmortem studies of SZ patients have revealed a loss of parvalbumin (PV) in the prefrontal cortex (PFC). Because PV expression is activity-dependent, this finding likely reflects alterations in the activity of the fast-spiking inhibitory interneurons that express PV. A diminution of GABAergic input from PV cells could disrupt the circuit level balance between excitation and inhibition (E/I) in ways that affect cognition. Here, we examine whether increasing the activity of prefrontal PV cells is sufficient to normalize E/I balance and rescue cognition in an animal model of SZ. To generate this model, we administer MK801, an NMDA antagonist, to adolescent rats, which reduces prefrontal PV levels and recapitulates many of the behavioral endophenotypes of SZ. To increase the activity of PV cells, we inject a virus into the PFC delivering a PV-promoter driven excitatory hM3Dq DREADD (PV-DREADD). Subsequent administration of CNO activates the PV-DREADD, increasing the activity of transfected PV cells. Our electrophysiological data indicate an elevated prefrontal E/I ratio in MK801-treated female rats, which is brought back to control levels by activation of the PV-DREADD. These electrophysiological findings are reflected in behavioral performance on two behavioral tests of cognition; MK801-treated female rats show impairments in working memory and cognitive flexibility compared to saline-treated controls, and performance improves with PV-DREADD activation. Therefore, upregulating the activity of prefrontal PV cells may be sufficient to rescue the cognitive deficits found in SZ. To further characterize the relationship between PV, MK801 treatment, and cognition, we compare PV cell counts in MK801- and saline-treated rats, and compare PV expression with task performance.

Disclosures: L. Chamberlin: None. **B.R. Ferguson:** None. **E.P. McEachern:** None. **W.J. Gao:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

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Topic: H.03. Schizophrenia

Support: R25 NS080685

U01 NS099577
R01 DC00566
R01 NS081248

Title: CaMKIIα KO and WT differed in their ability to learn in an olfactory learning task and working memory

Authors: *D. RAMIREZ-GORDILLO[1], L. MEDIAVILLO[3], A. OLSON[1], K. BAYER[2], D. RESTREPO[1];
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Abstract: Learning and memory are two crucial abilities that are important for survival of organisms. Our lives are enhanced due to the ability to learn and form memories that shapes our personalities. Therefore, it is not surprising that often diseases that affect these abilities are devastating and that the individual affected becomes dependent on others. The α-isoform of calcium/calmodulin-dependent protein kinase II (CaMKIIα) plays an important role in long term potentiation (synaptic plasticity) an essential process for learning and memory. Mutations to CaMKIIα increased the risk of developing a schizophrenia, a neuropsychiatric disorder characterized by impaired concentration, working memory, perception and social dysfunction. Heterozygous CaMKIIα knockout mice (Het) have been described to show a schizophrenia-related phenotype including immature dentate gyrus (DG), hyperactivity, working memory deficits, and social withdrawal. CaMKIIα is expressed in areas of the brain important for learning and memory including prefrontal cortex and hippocampus. The role of CaMKIIα in olfactory learning and working memory is not well understood. To further investigate the role of CaMKIIα in olfactory learning and working memory, we used a go-no go olfactory discrimination task and an olfactory working memory task to assess cognitive learning deficits and awake behaving tetrode recording to measure neuronal oscillations in the hippocampus and prefrontal cortex. Mice learned to associate an odorant with a water reward in the go-no go task. Mice received double tetrode implants aimed at the CA1 region of the hippocampus and medial prefrontal cortex. All mice learned to differentiate between dissimilar odors. However, when the odor pair was similar and was reversed, Het mice took longer to learn the task. Furthermore, Local Field Potential measurements indicate a difference in the oscillation power between the CaMKIIα KO and WT. We are exploring differences in performance between CaMKIIα Hets and wild type mice in an olfactory working memory task. These observations suggest a key role of CAMKIIα in associative odorant learning and working memory.

Disclosures: D. Ramirez-Gordillo: None. **L. Mediavillo:** None. **A. Olson:** None. **K. Bayer:** None. **D. Restrepo:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.17/AA13

Topic: H.03. Schizophrenia

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Title: Specific, modular correction of hippocampal deficits across murine disease models

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Abstract: Hippocampal circuit disruptions are characteristic of neurological and psychiatric diseases, generating disease-related symptoms and making them attractive therapeutic targets. The hippocampus' modular structure introduces interesting implications for managing symptoms. While structurally similar across the septotemporal axis, individual modules within the hippocampus support different behavioral functions. For example, the ventral CA1 critically supports social discrimination. In an experimental mouse model of 22q11.2 deletion syndrome (22q11.2DS), hyperactivity in the vCA1 disrupts social discrimination performance such that 22q11.2DS mice fail to distinguish novel from familiar conspecifics. Using either muscarinic or kappa-opioid inhibitory DREADDs, we were able to reduce the 22q11.2DS-associated hyperactivity to rescue social discrimination function. Similarly, an experimental mouse model of temporal lobe epilepsy (TLE) exhibits a hyperactive dorsal dentate gyrus, a critical circuit for spatial discrimination. TLE mice display compromised behavioral performance; however, reducing dorsal dentate activity with either inhibitory DREADD again restored behavioral function. Interestingly, overly recruiting the inhibitory DREADDs in either disease model compromised behavioral performance, indicating that tuning circuit activity to appropriate levels is critical for successful coding. Furthermore, DREADD-induced effects were module-specific: recruiting dorsally expressed DREADDs did not affect ventral hippocampal-dependent behavioral outcomes and vice-versa. These findings suggest that correcting inappropriate disease-associated circuit tuning is sufficient to restore circuit function. Targeting circuit activity in a specific, modular manner may prove an effective therapeutic approach.

Disclosures: **J.B. Kahn:** None. **R.G. Port:** None. **H. Takano:** None. **S.A. Anderson:** None. **D.A. Coulter:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

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Topic: H.03. Schizophrenia

Support: DFG Grant BA 1582/2-2

Title: Disrupted-in-schizophrenia-1 is required for proper prefrontal pyramidal-interneuron circuit architecture

Authors: *J.-F. SAUER, M. BARTOS;
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Abstract: Psychiatric disorders greatly impact life quality of affected individuals while treatment options remain limited. Both environmental and genetic risk factors contribute to psychiatric conditions but the molecular and network defects leading to psychiatric disease states are insufficiently understood. Here, we have investigated the prefrontal cortical network of a genetic mouse model of psychiatric disorders. We analyzed adult mice lacking functional Disrupted-in-Schizophrenia 1 (Disc1 mice), a model of a rare mutation with high predisposition to depression and schizophrenia in humans. Using multiple single-unit recordings from freely moving and head-fixed mice, we show that prefrontal glutamatergic as well as GABAergic neurons show reduced spike rates in Disc1 mice. To reveal the potential mechanisms underlying reduced neuronal activity levels, we focused on synaptic interactions between local pyramidal cells and GABAergic interneurons. These connections can be identified *in vivo* by cross-correlation of spike trains. We find reduced connectivity and spike transmission of pyramidal cells onto putative GABAergic interneurons identified by spike shape, suggesting impaired hardwiring and maintenance of synaptic efficacy in the prefrontal cortex of Disc1 mice. Furthermore, we specifically probed excitatory connections onto parvalbumin-positive interneurons (PVIs) identified by optogenetic activation of channelrhodopsin-2 selectively expressed in PVIs. In head-fixed mice navigating in a virtual reality, we show that spike transmission probability at pyramidal cell-PVI connections is significantly reduced in Disc1 mice, indicating diminished synaptic efficacy. Our data thus reveal a causal role of Disc1 in pyramidal cell-interneuron synapse function in the prefrontal cortex *in vivo*.

Disclosures: **J. Sauer:** None. **M. Bartos:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

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Topic: H.03. Schizophrenia

Title: NMDA receptors on parvalbumin-positive interneurons and pyramidal neurons both contribute to MK-801 induced gamma oscillatory disturbances: Complex relationships with behaviour

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Abstract: Background: NMDAr antagonists induce disturbances to gamma frequency oscillations akin to abnormalities reported in clinical EEG studies in patients with schizophrenia. Specifically, administration of NMDAr antagonists such as ketamine and MK-801 to rodents (1) increases background or ongoing gamma activity and (2) reduces stimulus-evoked gamma oscillations. We sought to investigate the role PV+ neurons and pyramidal cells play in NMDAr antagonist induced disturbances in gamma oscillatory activity and behaviour by utilising two transgenic mouse lines in which cell-type specific deletion of the obligatory N-methyl-d-aspartate receptor subunit 1 (NR1) in parvalbumin positive neurons (PV:NR1 KO mice) and pyramidal neurons (CaMKIIα:NR1 KO mice) was achieved.
Methods: Two separate cohorts of adult animals were used in this experiment: Cohort 1 [(PV:NR1 KO mice (n=26) and wild-type littermates (n=28)] and cohort 2 [(CaMKIIα:NR1 KO mice (n=25) and wild-type littermates (n=20)] were first used to assess behavioural outcomes. Specifically, PPI and locomotor activity were assessed following administration of the NMDAr antagonist MK-801 (0.3 - 1.0mg/kg) or vehicle (saline). Following behavioural assessment, mice were implanted with electrodes in the medial prefrontal cortex (mPFC) and dorsal hippocampus (dHPC) to allow for local field potential (LFP) measures to be obtained.
Results: In control mice, MK-801 increased ongoing gamma power, reduced auditory evoked gamma power and increased gamma band coherence between the mPFC and dHPC. Behaviourally, these changes were accompanied by hyperlocomotor activity as well as deficient PPI. These consequences of NMDAr antagonism were differentially regulated in PV:NR1 KO mice and CaMKIIα:NR1 KO. The MK-801induced increase in ongoing gamma power was significantly attenuated in both PV:NR1 KO mice and CaMKIIα:NR1 KO mice, but deficits to auditory evoked gamma activity were unaffected by genotype. Interestingly, in contrast to PV:NR1 KO mice, the emergence of abnormal gamma band hyperconnectivity between the mPFC and dHPC following MK-801 treatment was completely absent in CaMKIIα:NR1 KO mice.

Conclusion: The results of this study suggest that while disturbances to local gamma synchrony can be caused by NMDAr antagonism on both PV interneurons and pyramidal neurons, NMDAr blockade on pyramidal neurons is crucial for inducing disturbances to long-range coherence and regional communication.

Disclosures: M.R. Hudson: None. **E. Sokolenko:** None. **T.J. O'Brien:** None. **N.C. Jones:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

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Program #/Poster #: 087.20/AA16

Topic: H.03. Schizophrenia

Support: NIH grant NS104705

Title: Role of GluN2C subunit in nucleus reticularis of the thalamus in schizophrenia-like phenotypes in mice

Authors: *G. P. SHELKAR[1], J. LIU[1], F. ZHAO[2], R. PAVULURI[1], P. GANDHI[1], R. CLAUSEN[2], S. M. DRAVID[1];
[1]Pharmacol. and Neurosci., Creighton Univ., Omaha, NE; [2]Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Schizophrenia is a mental disorder with a wide spectrum of symptoms. NMDA hypofunction hypothesis is a center to the current thinking of schizophrenia pathogenesis. Indeed, acute pharmacological blockade of NMDA receptors induces positive, negative as well as cognitive symptoms of schizophrenia. The nucleus reticularis of the thalamus (nRT) is a sheet of inhibitory neurons that covers the thalamus at the anteroposterior extent and shown to play an important role in the pathogenesis of the schizophrenia. The glutamatergic collaterals to the nRT from the cortical pyramidal and thalamic relay neurons express NMDA receptors. We and others have shown enriched expression of GluN2C subunit in PV neurons in nRT, however, their functional role in regulating nRT function is not well understood. Herein, we took advantage of the novel highly potent GluN1/GluN2C NMDA receptors selective co-agonist, AICP to assess the functional role of GluN2C subunits in schizophrenia-like phenotypes. We found that intracerebroventricular administration of AICP did not affect basal locomotion or prepulse inhibition but facilitated MK-801-induced hyperlocomotion. This effect was observed in wildtype but not in GluN2C KO mice. AICP also affected nRT neuron function in a GluN2C-dependent manner. In order to further evaluate whether the *in vivo* effect of AICP is mediated through modulation of nRT neurons, we employed a chemogenetic approach. We stereotaxically injected AAV2/9-Syn-DIO-hM3D(Gq)-mCherry or AAV2/9-Syn-DIO-hM4D(Gi)-mCherry

DREADDs viral vectors into the nRT of PV-Cre mouse line. We found that similar to AICP effect, activation of Gq but not Gi-coupled DREADD facilitated MK-801-induced hyperlocomotion. In contrast, modulation of PV nRT neurons by DREADD only modestly affected the basal locomotor activity and did not affect basal PPI and startle amplitude. Together, these results suggest that modulation of nRT neurons does not affect basal locomotion, startle amplitude or PPI. Whereas, MK-801-induced hyperlocomotion may results from changes in nRT neuron and GluN2C-containing receptor function. Our studies also demonstrate *in vivo* targeting of GluN2C-containing NMDA receptors by AICP.

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Poster

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Topic: H.03. Schizophrenia

Support: Pritzker Neuropsychiatric Research Consortium
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Hope for Depression Research Foundation

Title: Human thalamus *in situ* hybridization. Variations in distribution and gene expression level of multiple probes between control subjects

Authors: *R. CALZAVARA[1], J. J. FITZPATRICK[1], J. D. BARCHAS[2], W. E. BUNNEY[3], F. S. LEE[2], R. M. MYERS[4], A. F. SCHATZBERG[5], H. AKIL[1], S. J. WATSON[1];
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Abstract: The thalamus is a key node in neural network for perception, attention, cognition, emotion and is fundamental for sleep/wake cycles. Not surprisingly, the thalamus is a complex structure of multiple nuclei which are anatomically and functionally distinct. Each nucleus includes populations of different cell type which are not well known. This complexity is greatly enhanced in primates and particularly in human. The goal of our study is to generate a molecular map of the human thalamus in order to identify potential differences in thalamic gene-expression in schizophrenia. We are analyzing the distribution and mRNA expression level of selective genes throughout the thalamus (a total of 28 probes) including markers of Calbindin and Parvalbumin projection neurons as well GABAergic and Neuropeptide neurons. In particular, we

aim to identify 1) thalamic region for connectivity with prefrontal cortex and 2) cell types distribution within cytoarchitectonically-defined nuclei. By using mRNA *in situ* hybridization throughout the extent of the human thalamus, we are systematically characterizing the impact of variables known to affect mRNA expression, including technical variables such as tissue handling and processing, and biological variables such as age, gender, cause of death (Li et al., 2004; Hagenauer et al., 2018). Given the importance of pH in the fresh-frozen human brain tissue, this variable is carefully controlled in our cohorts. Our findings suggest potential individual differences in thalamic gene expression between control subjects, in particular for some gene markers and thalamic nuclei, such as for the mRNA expression of CALB1 in the medio-dorsal nucleus. It is therefore important to recognize that there is a wider variation than expected. These variations point to our ability to capture individual differences even within the control population, and lay the groundwork for future comparisons between controls and pathological conditions such as schizophrenia.

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Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.22/AA18

Topic: H.03. Schizophrenia

Title: Changes in the interneuron network contribute to adolescent pathology in a 15q13.3 microdeletion mouse model

Authors: *B. SOMMER, N. SCHUELERT, S. JAEGER, S. HOBSON, H. ROSENBROCK, V. MACK, **M. ZONOUZI**;
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Abstract: Schizophrenia is a neurodevelopmental psychiatric syndrome, which affects about 1% of people during their lives. First symptoms typically manifest in adolescence, which coincides with a critical period of prefrontal cortex maturation. While previous studies have identified deficits in cortical interneuron integrity and network function in schizophrenia patients, little is known about the maladaptive circuitry in prodromal stages of the disease. To assess changes in the brain network during adolescence that might contribute to the disruption of cortical function we have studied a mouse model of a human copy number variant known to confer high risk for psychiatric disorders such as schizophrenia. The 15q13.3 microdeletion mouse (Df(15q13)-/+) was examined by slice electrophysiology and high content histology. We explored markers of cortical network synchrony and GABAergic interneuron function in the prefrontal cortex and limbic system during adolescence (PND35) and adulthood (PND70). The Df(15q13)-/+ mice showed robust deficits in connectivity of specific interneuron subtypes in key brain regions associated with schizophrenia. In addition, we revealed apparent differences in cortical and limbic pathophysiological characteristics between adolescence and adulthood. Taken together, these data indicate abnormal interneuron integration during critical periods of brain maturation as part of the developmental trajectories leading to schizophrenia related network disruptions and, eventually, provide insight into new therapeutic opportunities for early intervention.

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Poster

087. Schizophrenia: Animal Models and Genetic Studies

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Program #/Poster #: 087.23/AA19

Topic: H.03. Schizophrenia

Support: Brain & Behavior Research Foundation
Biogen Inc.
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Boston Undergraduate Research Opportunities Program

Title: Increased expression of schizophrenia-associated gene C4 leads to hypoconnectivity of prefrontal cortex and reduced social interaction

Authors: A. L. COMER[1], T. JINADASA[2], L. KRETSGE[1], T. NGUYEN[3], J. LEE[2], E. NEWMARK[2], F. HAUSMANN[2], S. N. ROSENTHAL[4], K. LIU KOT[2], W. YEN[3], *A. CRUZ-MARTIN[2];
[1]The Grad. Program for Neurosci., [2]Biol., [3]Biomed. Engin., Boston Univ., Boston, MA; [4]Biol., Connecticut Col., New London, CT

Abstract: It remains unclear how circuit wiring is orchestrated during development and how misregulation of specific steps in this process contribute to brain diseases. Studies in humans and mouse models of neurodevelopmental disorders have established the involvement of immune molecules in neuronal development and brain pathology. Specifically, complement proteins play a role in various stages of brain development including neurogenesis, migration and synaptic development and pruning. The immune gene complement 4 (C4), located in the MHC locus, is highly associated with schizophrenia such that specific structural variants and regulatory regions increase expression of the gene and confer greater risk for this brain disorder. It is not understood how increased expression of C4 could be mechanistically linked to aberrant circuit dysfunction in the medial prefrontal cortex, a brain region implicated in schizophrenia. Using gene transfer approaches, we studied the role of increased levels of C4 in the developmental wiring of excitatory neurons in the prefrontal cortex. We also examine how altered microglia-neuron interactions contribute to prefrontal cortex dysfunction. Lastly, we tested whether complement-induced changes to specific prefrontal cortex microcircuits are sufficient to cause deficits in social behaviors. Our findings implicate a previously unknown role for C4 in prefrontal cortex circuit dysfunction.

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Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.24/AA20

Topic: H.03. Schizophrenia

Title: The bacterial metabolite indole-3-propionic acid increases kynurenic acid levels in the rat brain *in vivo*

Authors: *T. BLANCO-AYALA[1,2], K. SATHYASAIKUMAR[1], A. KLAUSING[1], R. SCHWARCZ[1];
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Abstract: Alterations in the composition and function of the gut microbiota may be causally associated with a number of brain diseases. Products of tryptophan metabolism are often suggested to play a major role in this context. Indole-3-propionic acid (IPrA) is a tryptophan-derived metabolite, produced by intestinal commensal microbes (Wikoff et al., 2009). The compound is probably not metabolized further in the gut but can cross the blood-brain barrier. In humans, IPrA concentrations are normally in the micromolar range in the blood (Morita et al., 1992; Bansal et al., 2010) and in the low nanomolar range in cerebrospinal fluid (Young et al., 1980). Interestingly, IPrA has neuroprotective properties and has been proposed as a treatment for Alzheimer's disease (Bendheim et al., 2002). Connections between IPrA and the kynurenine pathway (KP), which accounts for ~95% of dietary tryptophan degradation in mammals and generates the neuroprotectant kynurenic acid (KYNA) among other metabolites, have not been considered so far. To examine this possible link experimentally, adult male Sprague-Dawley rats (250-300 g; n=6 per group) received a single oral dose of IPrA (200 mg/kg). Animals were euthanized after 90 min, 6 h or 24 h, and both IPrA and KYNA levels were measured in cortical tissue. Compared to vehicle-treated controls, which contained 0.7 ± 0.2 pmoles IPrA/mg tissue, IPrA levels at the three timepoints were 36.5 ± 6.0, 4.9 ± 0.9 and 1.2 ± 0.3 pmoles/mg tissue, respectively. KYNA levels were 17.1 ± 1.6, 10.3 ± 1.6 and 3.6 ± 0.6 fmoles/mg tissue after 90 min, 6 h and 24 h, respectively (controls: 5.5 ± 1.6 fmoles/mg tissue). Additional experiments using *in vivo* microdialysis in the rat striatum revealed a rapid and sustained ~2.5-fold elevation in extracellular KYNA levels in response to IPrA (200 mg/kg, p.o.) (n=5). Finally, the *de novo* production of [^{3}H]KYNA from [5-^{3}H]kynurenine within the striatum (Guidetti et al., 1995), determined 2 h after oral vehicle vs. IPrA (200 mg/kg) administration, was found to increase from ~3.7% to ~7.0% (n = 5 per group). Taken together, these results provide first evidence that the tryptophan-derived bacterial metabolite IPrA causes an increase in KYNA levels and neosynthesis in the brain. Modulation of tryptophan-derived bacterial products such as IPrA may therefore provide a novel strategy to affect brain KYNA function and dysfunction.

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Poster

087. Schizophrenia: Animal Models and Genetic Studies

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Topic: H.03. Schizophrenia

Support: USPHS grant P50-MH103222
NARSAD young investigator award

Title: Elevated kynurenic acid levels in the brain of germ-free mice

Authors: *F. M. NOTARANGELO[1], M. A. THOMAS[1], T. COKSAYGAN[2], L. J. DETOLLA[2], R. SCHWARCZ[1];
[1]Maryland Psychiatric Res. Center, Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Program of Comparative Medicine, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Mounting evidence indicates that the gut microbiota regulates brain functions and behavior. Notably, germ-free (GF) mice display deficits in memory tasks, suggesting that intestinal bacteria may play an important role in regulating cognitive functions. While the underlying mechanisms are complex and poorly understood, tryptophan and its metabolites may play a special role in this context since elevated levels of this essential amino acid are seen in the plasma of GF mice (Clarke et al., 2013). In mammals, dietary tryptophan is preferentially degraded via the kynurenine pathway (KP) to produce several neuroactive compounds, including kynurenic acid (KYNA), an antagonist of α7 nicotinic and NMDA receptors, 3-hydroxykynurenine (3-HK), a free radical generator, and quinolinic acid (QUIN), an NMDA receptor agonist. Using 2-3-month-old Taconic C57BL/6 GF and control mice (n = 8-10 per group), and measuring KP markers in brain and periphery, the present study was designed to investigate more broadly if the removal of gut microbiota affects KP metabolism. Our results confirmed the previously reported observation that the absence of gut microbiota increases tryptophan levels in the plasma of the host. We also found a significant reduction in kynurenine levels and a significant *increase* in KYNA, but no changes in QUIN levels, in the serum of GF mice. In the same animals, tryptophan 2,3-dioxygenase activity in the liver was significantly elevated. Compared to control animals, no significant differences in the tissue levels of tryptophan, kynurenine, 3-HK and QUIN were seen in the forebrain of GF mice. Interestingly, however, we detected a significant *increase* in KYNA levels in the brain (22.0 ± 3.0 fmol/mg protein in GF mice, 11.9 ± 1.4 fmol/mg protein in controls). These data suggest that the gut microbiota affects not only tryptophan levels in the host circulation but also influences - and possibly normally regulates - KP metabolite levels in the serum and, apparently specifically, controls brain levels of KYNA. By influencing the function/dysfunction of brain KYNA, the composition of the gut microbiome may therefore play a newly recognized role in cognitive processes in health and disease.

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Poster

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Topic: H.03. Schizophrenia

Support: MH103222

Title: The probiotic *lactobacillus reuteri* produces kynurenic acid from kynurenine *in vitro*

Authors: A. E. FOO, F. M. NOTARANGELO, ***R. SCHWARCZ**;
Maryland Psychiatric Res. Ctr., Baltimore, MD

Abstract: The gut-brain axis has recently emerged as a major topic of interest in psychiatry. Of special interest, studies in germ-free and antibiotic-treated rodents have demonstrated that intestinal bacteria may regulate cognitive functions and may therefore also play a role in the cognitive deficits which are often observed in psychiatric diseases. The kynurenine pathway (KP) of tryptophan degradation may constitute a molecular link in this respect, as increased circulating tryptophan levels have been reported in animals after the removal or during dysbiosis of gut microbiota (Kennedy et al., 2017). The KP contains several neuroactive compounds, including kynurenic acid (KYNA), an antagonist of N-methyl-D-aspartate and α7 nicotinic acetylcholine receptors, i.e. two receptors which are critically involved in cognitive processes. Notably, bacterial tryptophan metabolism can directly affect brain KYNA levels in the host (see Blanco-Ayala et al., this meeting), though the nature of the responsible bacteria remains unknown. As a recent study demonstrated that one bacterium, *Lactobacillus (L.) reuteri*, normalizes the impaired plasma levels of several KP metabolites in stressed mice (Marin et al., 2017), we now decided to examine its ability to produce KYNA *in vitro*. To this end, *L. reuteri* was cultured on MRS agar and then suspended in Hank's Balanced Salt Solution. Pilot studies were performed by incubating the bacterium in Hank's buffer at 37°C for 3 h in the presence of KYNA's immediate bioprecursor kynurenine (100 μM) to assess the *de novo* formation of KYNA by measuring the metabolite in the medium by HPLC. KYNA neosynthesis was unequivocally verified, and production increased linearly when bacterial concentrations were increased stepwise from 10^7 CFU/mL to 10^8 CFU/mL. In the following experiments, using 10^8 CFU/mL, we evaluated the dependency of the process on incubation time and on the concentration of kynurenine added to the assay mixture. KYNA production from kynurenine was linear over time (tested at 1, 2 and 3 h) and with kynurenine concentrations ranging from 10 μM to 1 mM. Notably, however, no KYNA neosynthesis was observed when the bacteria were incubated up to 3 h with 1 mM tryptophan. Ongoing experiments are designed to characterize the *regulation* of KYNA production by *L. reuteri* and, importantly, to test the hypothesis that manipulation of KYNA formation in the bacterium can predictably affect the presence and function of KYNA in the host brain *in vivo*.

Disclosures: A.E. Foo: None. **F.M. Notarangelo:** None. **R. Schwarcz:** None.

Poster

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Topic: H.03. Schizophrenia

Support: MH094445

Title: Nutrient sensing signaling in dorsolateral prefrontal cortex in schizophrenia

Authors: *A.-R. HAMOUD[1], R. E. MCCULLUMSMITH[2];
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Abstract: Schizophrenia affects about 1% of the US population, with hallucinations, psychosis and impaired cognition characteristic for the disorder. Currently there are no treatments available for the cognitive symptoms that are thought to be a result of dysfunction of excitatory neurotransmission. Impairment of excitatory synapses during development may lead to alterations in nutrient sensing signaling pathways that are necessary for normal neuronal function.
Previous studies have shown dysregulation in glucose uptake and utilization in the dorsolateral prefrontal cortex (DLPFC) as well as mitochondrial dysfunction and reduced expression of glycolytic enzymes. A key aspect of these metabolic pathways are the signaling proteins that regulate the expression of various glycolytic enzymes, glucose transporters and other proteins that contribute to energy uptake and utilization.
We hypothesize that metabolic dysregulation in schizophrenia is due to aberrant nutrient sensing signaling which contributes to impaired cognition.
We are studying expression and activity levels of several proteins that act as nutrient sensors in the brain: mTOR, LKB1, and O-linked N-acetylglucosamine (O-GlcNAc) Transferase.
Minibrain and postmortem tissue from schizophrenia (N=20) and control (N=20) will be used to characterize protein expression and activity. We hope to gain a better understanding of the changes in the pathways that govern nutrient signaling in schizophrenia to potentially develop treatments for this devastating illness.
Key words: Nutrients, signaling, minibrain, bioenergetics, metabolism, LKB1, mTOR, O-GlcNAc, Schizophrenia, postmortem, DLPFC

Disclosures: A. Hamoud: None. **R.E. McCullumsmith:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.28/AA24

Topic: H.03. Schizophrenia

Support: Mh107916

Title: Protein protein interaction of the PSD95 interactome

Authors: *R. ALNAFISAH[1], G. LABILLOY[3], J. REIGLE[3], K. GREIS[4], J. MELLER[4], R. E. MCCULLUMSMITH[1], A. FUNK[2];
[2]Neurosciences, [1]Univ. of Toledo, Toledo, OH; [3]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [4]Univ. of Cincinnati, Cincinnati, OH

Abstract: Background: Mounting genetic, proteomic, and biochemical evidence indicate rare mutations, abnormal protein-protein interactions, and altered synaptic signaling pathways may be at the root of the severe phenotypes seen in patients with schizophrenia. A major area yet to be fully elucidated is the understanding of the complex synaptic protein-protein interactions in normal and pathological conditions. Increasing focus has been directed toward the NMDAR and PSD-95 protein-protein interactomes, members of which are identified as high-yield risk factors for the development of schizophrenia. PSD-95 is the most abundant scaffolding protein in the PSD, with well characterized protein-protein interactions that modulate the trafficking of glutamate receptors and other PSD constituent proteins relevant for synaptic plasticity.
Methods: Magnetic dynabeads conjugated with anti-PSD-95 antibody were used to isolate PSD-95 protein complexes from 10 control and 10 schizophrenia dorsolateral prefrontal cortex samples. The complexes were eluted from the beads and processed for data-independent acquisition (DIA) LCMS/MS analysis on an ABSciex 5600+ mass spectrometer.
Results: Bioinformatic analyses revealed a signature of proteins that are dysregulated in synaptic PSD-95 interactome in schizophrenia. Pathway analysis show abnormalities in glycolysis and gluconeogenesis, p38 MAPK, and regulation of microtubule cytoskeleton signaling pathways. The top genes that are disrupted in schizophrenia 1433Ζ, rabphillin3a and collagen.
Conclusions: Our data reflect cutting-edge efforts in the field of psychiatry on schizophrenia research. These data indicate significant abnormalities of synaptic protein-protein interactions which indicate the disruption of important signaling, trafficking, and metabolic pathways for normal neurological function.

Disclosures: R. Alnafisah: None. **G. Labilloy:** None. **J. Reigle:** None. **K. Greis:** None. **J. Meller:** None. **R.E. McCullumsmith:** None. **A. Funk:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.29/AA25

Topic: H.03. Schizophrenia

Support: MH094445

Title: Microarray analysis in postmortem pyramidal neurons reveals molecular mechanisms in schizophrenia

Authors: *X. WU[1], R. SHUKLA[2], K. ALGANEM[1], J. REIGLE[3], M. S. SIMMONS[4], J. H. MEADOR-WOODRUFF[5], E. DEPASQUALE[6], J. MELLER[3], R. E. MCCULLUMSMITH[1]; [1]Univ. of Toledo, Toledo, OH; [2]Neurobio. of Aging and Depression, CAMH, Toronto, ON, Canada; [3]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [4]Psych-Behavioral Neurobiogy, UAB, Birmingham, AL; [5]Dept. of Psychiatry, Univ. Alabama at Birmingham, Birmingham, AL; [6]Univ. of Cincinnati, Cincinnati, OH

Abstract: Schizophrenia is a devastating disease that affects approximately 1% population worldwide. Despite decades of investigation, there has not been a recent breakthrough in clinical treatment for schizophrenia. The molecular mechanism of schizophrenia has been largely investigated, but most of the studies focused on the region level, rather than the cellular level. To fill this gap, we performed microarray assays on 2000 pyramidal neurons from both superficial and deep layers of anterior cingulate cortex obtained by laser capture microdissection from 12 schizophrenia and 12 control postmortem brains. Gene set enrichment analysis (GSEA) was applied to the entire pre-ranked differential expression gene lists to gain a complete pathway analysis throughout all annotated genes. Our analysis revealed over represented groups of gene sets particularly in energy metabolism and immunity related pathways, which appeared in the neurons of both superficial and deep layers, suggesting the disruption of these pathways plays an important role in schizophrenia pathology. In addition, we found out that the top 5 hits in the downregulated gene sets of superficial pyramidal neurons are all involved in olfactory sensing, possibly explaining the olfactory dysfunction observed in the clinic of schizophrenia patients. To confirm the pathway analysis from GSEA, we took the top 500 differentially expressed genes in the superficial and deep neurons and ran through pathway analysis tools Enrichr and PiNET to perform a more stringent analysis. Similar pathways were also the hits in these additional analyses, indicating the reliability of GSEA analysis. Integrative LINCS was used to identify small molecule chemical perturbagens and knockdown signatures to estimate possible signaling pathways. Our iLINCS analysis indicated dysfunctional glucose metabolism and altered immunity in schizophrenia patients, which is consistent with other analyses above. Taken

together, our microarray study revealed the possible molecular mechanisms of schizophrenia pathology and may provide small molecule targets that can be used for schizophrenia treatment.

Disclosures: X. Wu: None. **R. Shukla:** None. **K. Alganem:** None. **J. Reigle:** None. **M.S. Simmons:** None. **J.H. Meador-Woodruff:** None. **E. Depasquale:** None. **J. Meller:** None. **R.E. McCullumsmith:** None.

Poster

087. Schizophrenia: Animal Models and Genetic Studies

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 087.30/AA26

Topic: H.03. Schizophrenia

Support: MH094445

Title: Characterization of AKT3 in the anterior cingulate cortex in schizophrenia

Authors: *E. A. DEVINE, S. M. O'DONOVAN, R. E. MCCULLUMSMITH;
Univ. of Toledo Col. of Med., Toledo, OH

Abstract: Schizophrenia is a devastating neuropsychiatric disorder that affects approximately 1% of the world's population. Its pathology involves the dysregulation of bioenergetics including the disruption of glucose metabolism as well as changes in the AKT pathway. This decrease in glucose metabolism has been linked to cognitive deficits, a primary symptom of schizophrenia. AKT, a serinine/threonine protein kinase, has three isotypes: AKT1, AKT2, and AKT3. Of the three isotypes, AKT 3 is most abundant in the human brain, more specifically in neurons. Previous studies have shown an increase in AKT kinase activity in schizophrenia in the anterior cingulate cortex (ACC), however changes in expression of the different isotypes, and more specifically AKT3 have yet to be studied.
We hypothesize that alterations in AKT3 expression will lead to dysregulation of downstream bioenergetic pathways, contributing to cognitive deficits associated with schizophrenia.
Our preliminary data shows a significant increase in AKT3 mRNA expression of schizophrenia (n=20) compared to controls (n=20) at the region level in the ACC. We will characterize mRNA and protein expression of AKT3 at the cellular level in enriched populations of pyramidal neurons captured using laser microdissection. qPCR will be used to assay cell-level mRNA expression and automated western blotting will be used to analyze both region and cell-level protein expression. In summary, we will characterize AKT3 gene and protein expression levels in the ACC in schizophrenia.
Key words: postmortem, schizophrenia, AKT, transcription factors

Disclosures: E.A. Devine: None. **S.M. O'Donovan:** None. **R.E. McCullumsmith:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.01/AA27

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NRF-2016M3C7A1905383

Title: Pinpoint monitoring of glucocorticoid receptor transcriptional activity in the IL-PFC mouse brain

Authors: *S. HER[1], Y.-M. HAN[2];
[1]KBSI, Seoul, Korea, Republic of; [2]Korea Basic Sci. Inst., Seoul, Korea, Republic of

Abstract: Bioluminescence imaging has proven to be a highly sensitive technique for assessing *in vitro* transcriptional activity toward understanding gene regulation patterns; however, application of this technique is limited for brain research. In particular, the poor spatiotemporal resolution is a main hurdle for monitoring the dynamic changes of transcriptional activity in specific regions of the brain during longitudinal analysis of living animals. To overcome this limitation, in this study, we modified a lentivirus-based luciferase glucocorticoid receptor (GR) reporter by inserting destabilizing sequence genes, and then the reporter was stereotaxically injected in the mouse infralimbic prefrontal cortex (IL-PFC). Using this strategy, we could successfully pin-point and monitor the dynamic changes in GR activity in IL-PFC during normal stress adaptation. The modified reporter showed a 1.5-fold increase in temporal resolution for monitoring GR activity compared to the control with respect to the intra-individual coefficients of variation. This novel *in vivo* method has broad applications, as it is readily adaptable to different types of transcription factor arrays as well spanning wide target regions of the brain to other organs and tissues.

Disclosures: S. Her: None. **Y. Han:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.02/AA28

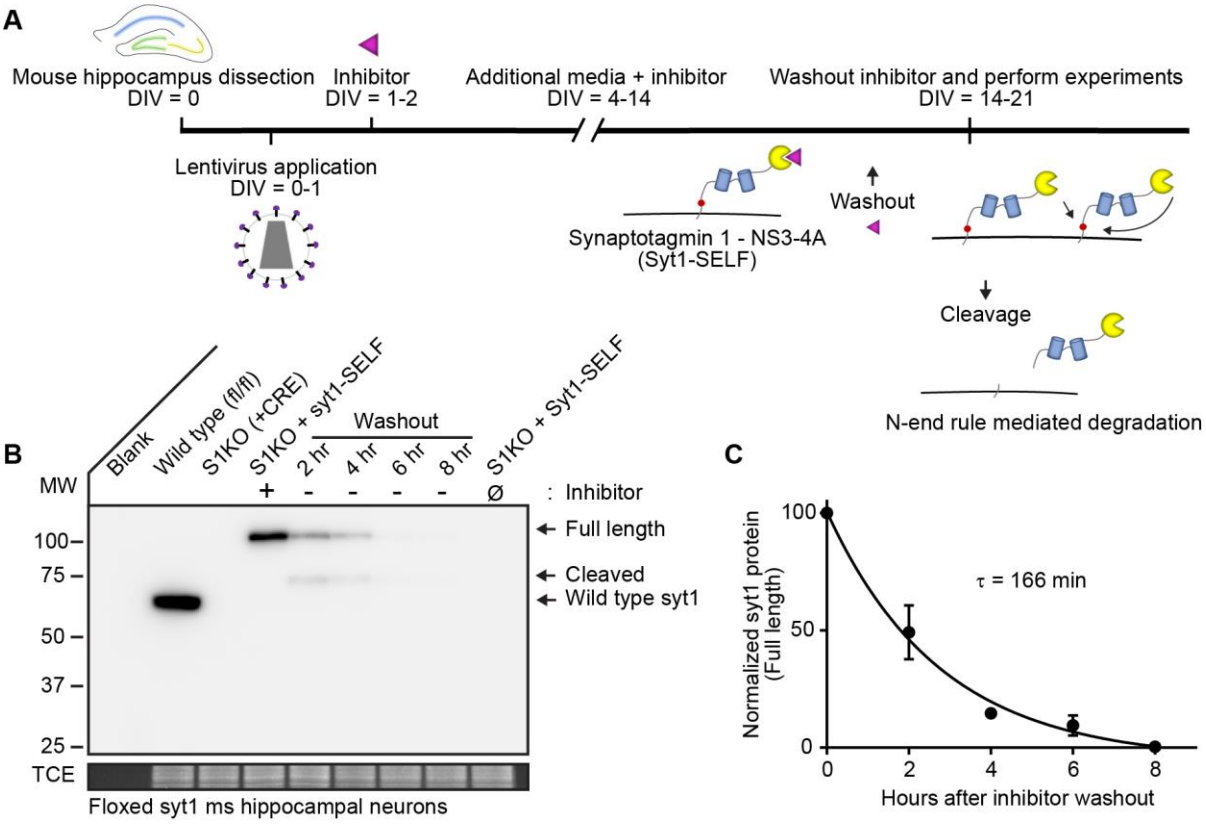
Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: F32 NS098604
NS097362
MH061876

Title: Knockoff: Acute disruption of a synaptic vesicle membrane protein (synaptotagmin 1)

Authors: *J. D. VEVEA[1], E. R. CHAPMAN[2];
[1]Univ. of Wisconsin - Madison HHMI, Madison, WI; [2]Neurosci., Howard Hughes Med. Inst., Madison, WI

Abstract: Here we describe knockoff, a generalizable method for the druggable control of integral membrane protein disruption. The protein of interest is tagged with the hepatitis C NS3/4A protease and is modified to contain a ten amino acid cleavage sequence. Control of cleavage is achieved through use of a clinically developed protease inhibitor. Washout of the inhibitor results in rapid cleavage and degradation of the protein of interest via the N-end rule. We developed knockoff for neuronal use and applied it to synaptotagmin 1 (syt1), a type 1 integral membrane protein. Acute disruption of syt1 using knockoff results in loss of synchronous neurotransmitter release and a concomitant increase in the spontaneous release rate. Thus, syt1 is not only the proximal Ca^{2+} sensor for fast neurotransmitter release, but also serves to clamp spontaneous release; the elevated spontaneous release rates observed in syt1 KO neurons are not the result of homeostatic plasticity.



(A) Illustration of the syt1 knockoff protocol. At 14-21 DIV, inhibitor is removed from neuronal

cultures and experiments are performed. Following inhibitor washout, the resulting cleavage product is degraded via the N-end rule. **(B)** Representative anti-syt1 immunoblot of WT, syt1 KO (generated using a CRE virus), and KO neurons expressing syt1-SELF, in mouse hippocampal neurons at 14 DIV. (Ø) denotes a condition in which cultures have never been exposed to inhibitor. **(C)** Self-cleavage time course of syt1-SELF, upon inhibitor washout. Cleavage was quantified via densitometry of the syt1 immunoblots in panel (B) and plotted. Mean +/- SEM from 3 trials are shown, and the time constant was determined by fitting the data with a single exponential function ($R^2 = 0.9498$). The τ for syt1-SELF cleavage was 2 hours 45 min.

Disclosures: J.D. Vevea: None. **E.R. Chapman:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.03/AA29

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Characterizing the neural progenitors and post-mitotic neurons using recombinant rabbit monoclonal antibodies

Authors: *S. BALASUBRAMANIAN[1], J. K. SUKUMARAN[2], S. SHANKAR[2], V. SREERAG[2], S. MENON[2], S. SAJJA[2], C. BHAMBHANI[2];
[2]R&D, [1]Thermo Fisher Scientific, Bangalore, India

Abstract: Recent advances in the field of stem cell biology along with enhanced differentiation protocols and reagents for specific lineages have enabled researchers to access differentiated cells that are otherwise challenging to obtain. This is especially true in the case of neuronal cells, where highly specific neurons are difficult to isolate and maintain in the lab and where cell banks provide only a select few immortalized neuronal cell lines. The applicability of these specialized cell populations ranges from their ability to facilitate neurodegeneration research to drug discovery and diagnostics through the identification of biomarkers for various applications. Given their importance, we offer several specific and extensively validated antibodies for important markers of pluripotency and neuronal differentiation. Here, we discuss some data from selected antibodies. Antibodies against neuronal targets were tested in the most appropriate neural populations which have been differentiated from iPSCs and NSCs. For example, antibodies against Nestin and SOX2 were validated in neural progenitors and neural rosettes; beta III tubulin and MAP2 in mature neurons; GFAP in astrocytes; Sox10 in Schwann cell progenitor. The data here shows that an antibody for any given cell type marker was tested in the appropriate application and in the right biological context. Induced pluripotent stem cells (iPSCs) differentiated along various lineages serve as powerful tools in establishing antibody specificity

against the intended protein specific to a particular cell type. In addition, we employ other relevant models to verify specificity. For example, the antibody against OTX2, a transcription factor that plays a major role in the brain and sensory organ development, was tested in retinal sections to check the specificity of the antibody by tissue immunofluorescence. Antibody specificity was also addressed at the immunogen design stage while developing an antibody. When the target antigen is highly homologous to multiple proteins, antigen design plays a crucial role in determining specificity of the antibody. Full length optineurin protein could not be used as immunogen owing to its homology with other mitochondrial proteins. Therefore, we designed two short critical regions of the protein to serve as immunogen that provided greater immunogenicity and specificity. In summary, we demonstrate systematic antibody validation strategies through a holistic understanding of the target protein biology.

Disclosures: S. Balasubramanian: None. **J. K. Sukumaran:** None. **S. Shankar:** None. **V. Sreerag:** None. **S. Menon:** None. **S. Sajja:** None. **C. Bhambhani:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.04/AA30

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: Alzheimer's Center grant, P30 AG035982

Title: Detection of mitochondrial DNA methylation and the effect of mitochondrial DNA-depletion on the nuclear DNA methylation pattern

Authors: *X. WANG[1], I. WEIDLING[1], H. M. WILKINS[2], S. KOPPEL[1], B. MENTA[1], J. PEREZ-ORTIZ[1], R. SWERDLOW[1];
[1]Univ. of Kansas Med. Ctr., Kansas city, KS; [2]Neurol., Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: DNA methylation is a critical epigenetic marker in the mammalian genome. Conflicting results were reported regarding the presence of methylated cytosines within mitochondrial DNA (mtDNA). To clarify this point, we applied Targeted Next-Generation Bisulfite Sequencing to examine the methylation status of highly purified mtDNA isolated from SY5Y cells. The detected regions covered 22 CpG sites across mtDNA D-loop region. We found that the average methylation level of mitochondrial D-loop was less than 4%. Meanwhile, significant false-positive DNA methylation was detected when sequencing template was amplified from bisulfite-converted genomic DNA instead of purified mtDNA. In addition, we observed the expression of DNA methyltransferases within the mitochondria. Moreover, we found that the COX IV-1 promoter was hyper-methylated in mitochondrial DNA-deficient SY5Y

ρ0 cells. Meanwhile, the absence of mitochondrial DNA induced the reduction of transcription and expression of COX IV-1 gene. In conclusion, our findings show that DNA methylation occurs in human mtDNA D-loop region and mtDNA depletion results in methylation dependent silencing of nuclear-encoded mitochondrial gene.

Disclosures: X. Wang: None. **I. Weidling:** None. **H.M. Wilkins:** None. **S. Koppel:** None. **B. Menta:** None. **J. Perez-Ortiz:** None. **R. Swerdlow:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.05/AA31

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH NS091585, NS085568
VA Merit Award RX000666, RX001473
AHA Award CDA34110317

Title: Chemical preconditioning of rat bone marrow mesenchymal stromal cells using a prolyl hydroxylase inhibitor for cell transplantation therapy after stroke

Authors: J. ZHAO, *Z. Z. WEI, M. QU, T. YU, S. YU, L. WEI;
Anesthesiol./ Neurol., Emory Univ. Sch. Med., Atlanta, GA

Abstract: Cell transplantation therapy using bone marrow mesenchymal stromal cell (BMSC) is a promising treatment for brain injuries such as ischemic stroke. Our group and others showed that hypoxic preconditioning of cells before transplantation could enhance cell survival and therapeutic benefits following transplantation into the ischemic brain. In the present investigation, we tested a chemical preconditioning using prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) to mimic a hypoxic condition to prime BMSCs before transplantation. BMSCs from postnatal Day 21 Wistar rats were cultured and maintained five passages before exposure to DMOG at 0.001, 0.1, 2, 10, or 50 mM for 24 or 48 hours. Stress factors in BMSC cultures including HIF-1alpha and its downstream genes erythropoietin, eNOS, PDK-1 were measured. DMOG at 10 mM for 48 hrs showed the highest increase of HIF-1alpha and the lowest cleaved caspase-3 level in BMSCs. Regular or DMOG pre-treated BMSCs were transplanted via the intranasal route 7 days after focal ischemic stroke in mice. Western blot analysis showed that, compared to regular BMSCs, transplantation of DMOG-preconditioned BMSCs increased angiogenic factors including HIF-1alpha, VEGF, and Glut-1, reduced apoptotic and inflammatory factors including the Bcl-2/Bax ratio, caspase-3, IL-1beta, IL-6, and IL-10. Transplantation of DMOG-BMSCs also regulated the Wnt/beta-catenin signaling pathway. The NeuN+BrdU+ colabelled cells in the ischemic cortex were also significantly

higher in mice received DMOG-BMSCs. Preliminary data also suggest regulations of the oxytocin receptor in stroke mice received different BMSCs. Ongoing experiments are testing cognitive and social behavioral functions after stroke and treatments with different BMSCs. This study will provide the evidence for feasibility and efficacy as well as potential mechanisms in preconditioning BMSCs using a prolyl hydroxylase inhibitor for improved regenerative treatments after ischemic stroke.

Disclosures: **J. Zhao:** None. **Z.Z. Wei:** None. **M. Qu:** None. **T. Yu:** None. **S. Yu:** None. **L. Wei:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.06/AA32

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: University of Michigan

Title: Tools to monitor the activation of opioid receptor signaling pathways for screening drugs and detecting endorphin release

Authors: *K. KRONING, W. WANG;
Chem., Univ. of Michigan, Ann Arbor, MI

Abstract: Opioid medications are designed to treat pain by activating opioid receptors. The activation of these receptors leads to downstream signaling pathways, via recruitment of the heterotrimeric G-protein or beta-arrestin. G-protein signaling leads to analgesia, while beta-arrestin signaling has been linked to respiratory suppression and constipation. There is a need to design medications that produce the analgesic response of opioid signaling and not the negative side-effects associated with beta-arrestin signaling. To solely produce the analgesic response of opioid signaling, specific agonists that would biasedly stimulate the G-protein pathway must be identified. We developed a technique to select biased agonists that will cause the recruitment of the heterotrimeric G-protein to the receptor and not beta-arrestin, therefore preventing beta-arrestin signaling. This technique is a modification of a previously developed system for studying protein-protein interactions named **S**pecific **P**rotein **A**ssociation tool giving transcriptional **R**eadout with rapid **K**inetics (SPARK).[1] Our method is a reporter-based assay where a specific fluorescent protein readout will indicate which protein interacts with the receptor, beta-arrestin or the G protein. The cells expressing the fluorescent protein that indicates the G-protein recruitment can be screened using 384 well plates and plate readers.
1. Kim, M., Wang, W., Sanchez, M., Coukos, R., Zastrow, M., Ting, A. (2017) eLIFE. 6:e30233

Disclosures: **K. Kroning:** None. **W. Wang:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.07/AA33

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH Grant NS101620
NIH Grant EB022788

Title: Lysosomal cell death: Specific detection by simultaneous labeling of its nucleolytic and proteolytic markers

Authors: *V. V. DIDENKO[1,2], C. L. MINCHEW[1,2];
[1]Baylor Col. of Med., Houston, TX; [2]Michael E. DeBakey Veterans Affairs Med. Ctr., Houston, TX

Abstract: Lysosomes contain hydrolytic enzymes degrading proteins and DNA. Leakage of these reactive compounds through compromised lysosomal membranes causes lysosomal cell death. This type of cell death is particularly difficult to determine, as it can have apoptotic, necrotic or mixed morphology. Lysosomal cathepsin proteases, such as cathepsin D, and the lysosomal endonuclease, DNase II, have both been implicated in lysosome-related cell death. Here we present a highly specific dual-labeling technique for simultaneous visualization of these two lysosomal cell death markers. The approach labels the intracellular distribution of cathepsin D and the sites with DNase II-type breaks in fixed tissue sections. It determines the lysosomal or extra-lysosomal localization of the markers and can be useful in studying pathways and signals of lysosomal cell death.

Disclosures: **V.V. Didenko:** None. **C.L. Minchew:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.08/AA34

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Engineering REST/NRSF-specific synthetic PUF proteins to control neuronal gene expression: A combined experimental and computational study

Authors: *A. MEROLLA[1,2], S. CRISCUOLO[1], M. GATTI IOU[1], L. MARAGLIANO[1], F. CESCA[1,3], F. BENFENATI[1,4];
[1]Ctr. for Synaptic Neurosci., Italian Inst. of Technol., Genova, Italy; [2]Dept. of Exptl. Med., Univ. of Genova, Genova, Italy; [3]Dept. of Life Sci., Univ. of Trieste, Trieste, Italy; [4]IRCSS, Ospedale Policlinico San Martino, Genova, Italy

Abstract: ***Aims:*** RE1-silencing transcription factor (REST) is a transcriptional repressor that plays a key role in regulating nervous system development. Dysregulation of REST protein levels has been identified in several neuropathologies such as cerebral ischemia, Down syndrome, epilepsy, Huntington's disease and Alzheimer's disease. In order to promote the increase of REST expression in pathologies in which REST expression is low, we used a synthetic approach based on the engineering of REST-specific RNA binding *Pumilio and FBF* (PUF) proteins. PUFs bind RNA following a known recognition code, whereby each ribonucleotide is recognized by a protein motif containing five variable residues. By following this code we modified wild type PUF to make it specific for a sequence in the 3' UTR of REST mRNA. Computational modelling was also performed to investigate the molecular basis of PUF-RNA interaction.
Methods: REST-specific eight- and sixteen-repeat PUF proteins were built via site-directed mutagenesis. To test the specificity of the engineered constructs, electrophoretic mobility shift RNA assays and cross-linking RNA immunoprecipitation were used in vitro. PUF-RNA interactions were studied in silico by molecular dynamics simulations using the available crystal structure of the eight repeat PUF, and a model built by us for the sixteen repeat PUF.
Results: The sixteen repeat REST-specific PUF shows the highest specificity, and binds endogenous REST mRNA without altering its stability. Computational work shows that the PUF conformation depends on the presence of bound RNA and confirmed the importance of the recognition code for a stable interaction.
Conclusions:*The developed PUF-based probes will serve as a platform to anchor specific effector proteins to REST mRNA with the purpose to increase its stability.*

Disclosures: A. Merolla: None. **S. Criscuolo:** None. **M. Gatti Iou:** None. **L. Maragliano:** None. **F. Cesca:** None. **F. Benfenati:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.09/AA35

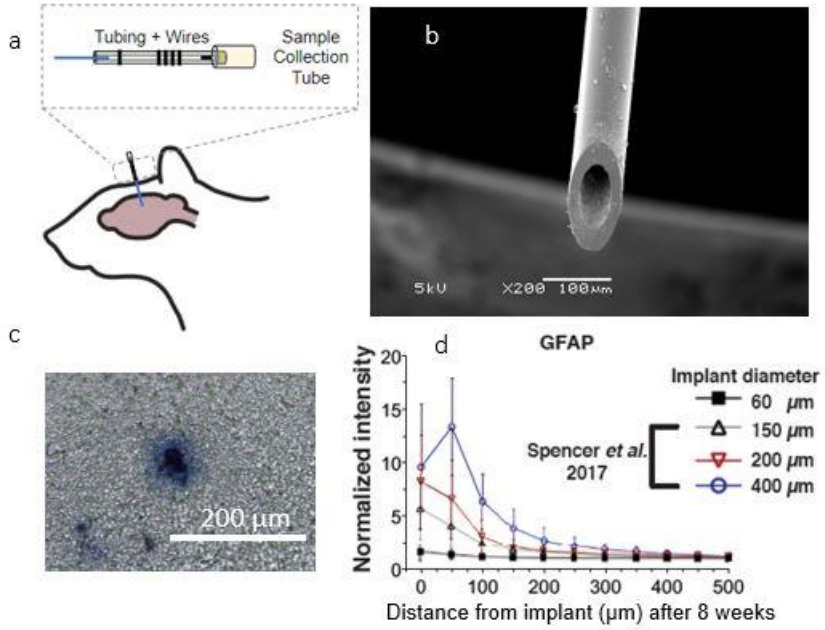
Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH R01 EB016101
NSF 2016220817

Title: Micro-invasive sampling platform for chronic detection of proteins and neuropeptides in brain interstitial fluid

Authors: *E. B. ROUSSEAU[1], R. RAMAN[2], M. J. COTLER[1], K. B. RAMADI[1], F. M. WHITE[3], R. LANGER[2], M. J. CIMA[4];
[1]Havard-MIT Hlth. Sci. and Technol., [2]Koch Inst. For Integrative Cancer Res., [3]Dept. of Biol. Engin., [4]Dept. of Material Sci., MIT, Cambridge, MA

Abstract: Understating the role neuropeptides play in pathology requires tools capable of chronic recording of region-specific dysregulation. Microdialysis enables the collection of small neurochemicals from interstitial fluid (ISF) via diffusion across a semipermeable membrane. Microdialysis has provided valuable insight into neurotransmitter function, but has significant limitations in neuropeptide sampling. Large probe sizes (>150 μm) limit spatial resolution and membranes are prone to fouling, limiting chronic functionality. Here we present a platform capable of low-flow fluidic delivery and membrane-free neurochemical sampling from ISF. This approach enables chronic recording of relatively large neuropeptides, proteins, and extracellular vesicles. The micro-invasive sampling platform consists of a polished borosilicate capillary (80 μm OD, 50 μm ID) interfaced with a custom peristaltic pump (nanopump) (fig a,b). The nanopump is capable of infusing at flow rates down to 1 nL/sec. Fluidic delivery was assessed by 30-60 second dye infusions into *ex vivo* brain tissue and examined by histology and tissue fluorescence (fig c). ISF samples were taken by infusing artificial CSF and reversing the nanopump's peristalsis to withdraw the infused bolus. Single cannula push-pull reduces overall device footprint, which leads to less scarring following chronic implantation (fig d). We investigated degradation of neuropeptides in CSF and found stability up to one month after snap freezing. Samples acquired via the sampling platform were acidified, desalted and solubilized. Processed samples were analyzed on a Thermo QExactive Orbitrap mass spectrometer. Detection of abundant brain proteins (Ubiquitin C-terminal hydrolase L1) demonstrated proof of ISF sampling. Building on our protocol for sampling ISF from *ex vivo* rodent brains, we are currently sampling, both acutely and chronically, in a rodent model. Smaller device footprints, low flow infusion and withdrawal rates, and a membrane-free single cannula system will allow for less disruptive, chronic neuropeptide monitoring.



Disclosures: **E.B. Rousseau:** None. **R. Raman:** None. **M.J. Cotler:** None. **K.B. Ramadi:** None. **F.M. White:** None. **R. Langer:** None. **M.J. Cima:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.10/AA36

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: Canadian Institutes of Health Research – Foundation Grant #353649

Title: Use of new lipid nanoparticule formulations for *in vivo* delivery of messenger RNA in the central nervous system

Authors: *K. NESZVECSKO[1], M.-A. DANSEREAU[2], J. CÔTÉ[3], J.-M. LONGPRÉ[4], P. SARRET[5];
[1]Inst. De Pharmacologie - Univ. De Sherbroo, Sherbrooke, QC, Canada; [2]Univ. De Sherbrooke, Sherbrooke, QC, Canada; [4]Pharmacol. and Physiol., [3]Univ. de Sherbrooke, Sherbrooke, QC, Canada; [5]Pharmacol. & Physiol., Univ. of Sherbrooke, Sherbrooke, QC, Canada

Abstract: Gene-therapy and RNA-based drugs represent emerging therapeutic avenues to overcome gene or protein dysfunction in pathological conditions. While most clinical trials using RNA currently focus on the use of short interfering RNA and antisense oligonucleotides to reduce the expression of a specific target, overexpressing a protein of interest by using

exogenous mRNA still remains an unmet need and challenge. In this study, we thus evaluated the ability of four new lipidic nanoparticle formulations (LNPs), conceptualized by Precision NanoSystems Inc., to increase delivery and overexpression of the encapsulated mRNA for the green fluorescent protein (GFP) in rats in vivo. We first injected intrathecally (i.t.) the four different LNP formulations (codenamed A, B, C and D) containing GFP-mRNA at the L4-L6 lumbar spinal segment and collected the corresponding dorsal root ganglia (DRGs) 6 hours later for further quantification of GFP expression by ELISA. GFP expression for both formulations A and B (160 and 45 pg/ml, respectively) were significantly higher than the negative control (11 pg/ml), while formulations C and D (13 and 15 pg/ml) were not different from the control conditions. A diffuse intracellular GFP fluorescent staining was also observed over DRG neuron cell bodies by confocal microscopy 6 hours post-injection. Kinetics of GFP protein expression was then evaluated with formulation A for up to 20 hours to determine the optimal expression time period in sensory neurons. We observed a time-dependent increase in GFP expression up to 6 hours followed by a decrease 20 hours post-injection. In order to assess supraspinal diffusion of our LNPs containing GFP mRNA, we further performed intracerebral injection of formulation A in the region of the nucleus raphe magnus. Brains were harvested 6 hours post-injection and GFP expression was detected by epifluorescence microscopy, proximal to the injection site, with a clear colocalization with the lipophilic dye contained in LNPs. The only adverse effect observed following i.t. injection of LNP formulations was a significant weight loss (up to 5.4%), independently of the formulation used. To better understand this observation, we assessed the activation of the immune system with ELISA quantification of pro-inflammatory cytokine IL-6 in protein extracts from DRGs. We found no apparent signs of inflammation at 3, 6 and 20-hours post-LNP injections. In conclusion, our results demonstrate that mRNAs encapsulated in a new LNP formulation rapidly translate in sustained production of a protein following spinal or brain delivery, thus opening the way to delivery of therapeutic-designed mRNA in pathological conditions.

Disclosures: K. Neszvecsko: None. **M. Dansereau:** None. **J. Côté:** None. **J. Longpré:** None. **P. Sarret:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.11/AA37

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NSERC

Title: Differential dorsal-ventral immediate early gene expression in CA1 during place navigation and contextual fear discrimination in rats

Authors: *E. D. MORGAN[1], R. MCHUGH[1], J. Q. LEE[1], R. J. SUTHERLAND[2], R. J. MCDONALD[3];
[1]Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Univ. Lethbridge, Lethbridge Alberta, AB, Canada; [3]Dept. of Neurosci., Univ. Lethbridge, Lethbridge, AB, Canada

Abstract: A growing body of evidence suggests that the dorsal and ventral hippocampus have different roles in memory-guided behaviour. The dorsal hippocampus has been suggested to be critical for representing precise spatial information, whereas the ventral hippocampus more strongly represents emotional and contextual features in long-term memory. Here, we report new findings that the immediate early gene Arc is expressed more strongly in dorsal than ventral CA1 during navigation in the Morris water task. We further explored differences in dorsal-ventral CA1 activation by probing cFos protein expression in the Morris water task and context fear discrimination in rats matched for experience in behavioural training and testing. The results of our experiments add to a growing literature on the possible roles of dorsal and ventral hippocampus in long-term memory and task performance. Future work will clarify whether these differences are related more to the content or the granularity of memory representation along the dorsal-ventral hippocampal axis.

Disclosures: E.D. Morgan: None. **R. McHugh:** None. **J.Q. Lee:** None. **R.J. Sutherland:** None. **R.J. McDonald:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.12/AA38

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Antisense oligonucleotides target neurons, oligodendrocytes, astrocytes and microglia in the rat central nervous system after intrathecal administration

Authors: *C. MAZUR, B. DEBROSSE-SERRA, H. T. ZHAO, B. POWERS, F. KAMME, J. WATSON, H. B. KORDASIEWICZ, E. SWAYZE;
Ionis Pharmaceuticals, Inc., Carlsbad, CA

Abstract: The central nervous system (CNS) is highly complex with different cell types interacting together. Ionis Pharmaceuticals develops antisense oligonucleotides (ASOs) as therapeutic molecules. These ASOs are large (6-8 kD) and highly charged, therefore they do not cross the blood brain barrier (BBB). In order to develop ASOs for neurological diseases, they must be delivered directly to the CNS to bypass the BBB. This is accomplished in the clinic for the ASO drug Spinraza by intrathecal (IT) administration utilizing lumbar puncture. The clinical administration of ASOs to the IT space is modeled in the rat by bolus injection via catheter

implanted into the lumbar spine.We wanted to examine the cell type distribution and pharmacology of ASOs in the rat after IT administration. We identified oligonucleotide fluorescence *in situ* hybridization (FISH) probes (Advanced Cell Diagnostics) for cell markers for the four major cell types in the CNS; neurons, astrocytes, oligodendrocytes and microglia. We also developed an ASO and a FISH probe for the long noncoding RNA Malat1 in the rat. We then performed an IT dose response of the Malat1 ASO in the rat and euthanized the animals 2 weeks following dosing. The CNS tissues were analyzed using multiplexed FISH for a CNS cell type combined with Malat1 and DAPI to visualize nuclei. The fluorescent signal for the Malat1 FISH probe was quantified within the different CNS cell types within gross structures of the CNS to determine the dose dependent reduction of Malat1 RNA expression in each cell population due to the pharmacological action of the Malat1 ASO. Dose response curves were compared between the different cell types in the different CNS structures. Analysis of the multiplexed FISH data demonstrated that the ASO was distributed to and reduced the Malat1 RNA expression in all the CNS cell types evaluated. We then went on to confirm the cell type specific effects of ASO by cell type isolation and PCR and by reduction of targets whose expression is restricted to the individual cell types.

Disclosures: **C. Mazur:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. 2855 Gazelle Court, Carlsbad, USA. **B. DeBrosse-Serra:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. Carlsbad CA USA. **H.T. Zhao:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. Carlsbad CA USA. **B. Powers:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. Carlsbad CA USA. **F. Kamme:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. Carlsbad CA USA. **J. Watson:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. Carlsbad CA USA. **H.B. Kordasiewicz:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. Carlsbad CA USA. **E. Swayze:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals, Inc. Carlsbad CA USA.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.13/AA39

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: ZooMAbs- The next generation of recombinant monoclonal antibodies

Authors: *W. A. SPECKMANN, C. MOHAN, Y. JAN, W. ZHENG;
MilliporeSigma, Temecula, CA

Abstract: Antibodies have become one of the most important tools in life science research, allowing the detection, quantitation, and determination of changes in proteins and other

molecules with respect to time and subcellular location. MilliporeSigma's ZooMAb™ antibodies represent a new generation of monoclonal antibodies that are specifically engineered using proprietary technologies that provide state-of-the-art consistency and applications performance. ZooMAbs™ are designed with the most user-friendly formulation, handling, and storage features available today and have been validated in multiple immunoassay applications. This platform acquired its unique name because of its proprietary B cell immortalization technology that also allows us to produce monoclonal antibodies from a wide range of species including rabbits, goats, llama, sheep, and more. The monoclonal antibodies produced and selected are subsequently converted into a eukaryotic recombinant expression format. Thus, all ZooMAb™ antibodies are offered as affinity purified recombinant proteins for highest reproducibility, purity, and performance. In this poster, we will highlight the development process of ZooMAbs™, show examples of the outstanding affinity, avidity, and specificity of these antibodies in multiple immunoassays, provide details about the earth friendly format they are provided in, and finally details about future directions as well as custom development opportunities will be discussed.

Disclosures: **W.A. Speckmann:** A. Employment/Salary (full or part-time):; MilliporeSigma. **C. Mohan:** A. Employment/Salary (full or part-time):; MilliporeSigma. **Y. Jan:** A. Employment/Salary (full or part-time):; MilliporeSigma. **W. Zheng:** A. Employment/Salary (full or part-time):; MilliporeSigma.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.14/AA40

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: Neuroscience program
College of Medicine
Department of Chemistry and Biochemistry
Field Neurosciences Institute
John G Kulhavi Professorship in Neuroscience at CMU

Title: *In vitro* uptake, delivery, and temporal regulation of hBDNF via surface modified PAMAM dendrimer nanoparticles using TET system

Authors: *C. E. THOMPSON[1,2], P. OTERO[1,2], B. SRINAGESHWAR[3,2,4], M. FLORENDO[2,4], A. SHARMA[5], A. M. FIGACZ[4], R. A. KIM[4], D. SWANSON[5], G. L. DUNBAR[6], J. ROSSIGNOL[7];
[1]Field Neurosciences Inst. Lab. for Restorative Neurol., [2]Program in Neurosci., Central Michigan Univ., Mount Pleasant, MI; [3]Neurosci., Central Michigan Univ., Chennai, India; [4]Col. of Med.,

[5]Dept. of Chem. and Biochem., [6]Neurosci., Central Michigan Univ., Mount Pleasant, MI; [7]Field Neurosciences Inst. Lab., Mount Pleasant, MI

Abstract: Huntington's disease (HD) is a neurodegenerative disease causing cognitive, mood, and motor dysfunction in patients. Studies have shown that levels of brain derived neurotrophic factors (BDNF), known for maintenance and survival of neurons, is reduced in individuals with HD. However, an excess of BDNF, which can result from transplanting cells that overexpress this protein, the motor dysfunction can be exacerbated.. To regulate BDNF production following transplantation, a complex of human BDNF (hBDNF) and G4 PAMAM 90/10 dendrimers were created to be used with the tetracycline-ON (TET ON) system to control gene expression. PAMAM dendrimers are nanoparticles synthesized with: (1) a DAB core; (2) a generation-variable, three- dimensional branching system; and (3) a 100% amine surface. Dendrimers can be easily taken up by cells and pass through the blood-brain barrier (BBB). However, most dendrimers contain a 100% amine surface, which is toxic and results in cell death. To create our complex, we synthesized a G4 PAMAM 90% hydroxyl and 10% amine surface (90/10) dendrimer to reduce toxicity. Then, the complex of dendrimer and plasmid was created and analyzed using gel electrophoresis. Complexes with the TET plasmid, were formed which contained reporter genes. The complex was added to HEK293 cells. We then observed the cells for: (1) complex uptake; (2) cell viability via MTT assay; and (3) regulation and production of hBDNF using ELISA, which was shown after addition of doxycycline (DOX) under fluorescence microscopy. Our findings indicate the TET ON system was able to regulate the expression of hBDNF *in vitro* with high cell viability. This system of dendrimer-plasmid delivery, combined with TET ON could be studied further *in vivo*, and in the future could be used to move plasmids past the BBB and regulate hBDNF production, providing a non invasive treatment for neurodegenerative diseases, such as HD..

Disclosures: C.E. Thompson: None. **P. Otero:** None. **B. Srinageshwar:** None. **M. Florendo:** None. **A. Sharma:** None. **A.M. Figacz:** None. **R.A. Kim:** None. **D. Swanson:** None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.15/DP13/AA41

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Precise morphological mapping of high plex neurodegenerative and neuroinflammation protein targets in human FFPE brain tissue with digital spatial profiling

Authors: A. ROSENBLOOM[1], A. BAHRAMI[1], L. BOGATZKI[1], W. CARTER[1], *J. R. KUHAR[2], K. R. MILLER[2], Y. LIANG[1], G. GEISS[1], J. M. BEECHEM[1];
[1]Nanostring Technologies, Inc, Seattle, WA; [2]Nanostring Technologies, Seattle, WA

Abstract: Neurodegenerative diseases, including Alzheimer's, Parkinson's, and amytrophic lateral sclerosis (ALS), affect millions of people worldwide and represent an increasing burden in terms of healthcare economics and societal impact. The investigation and characterization of the abundance, specific spatial distribution and co-localization of neurodegenerative and neuroinflammatory targets within the human brain are critical for the advancement of our understanding of progressive degenerative disease. Nanostring's Digital Spatial Profiling (DSP) technology allows for simultaneous analysis of >40 proteins from discrete regions of interest (ROI) in formalin fixed paraffin-embedded (FFPE) tissue sections, providing morphological context to high plex molecular analysis. The assay relies upon antibody probes coupled to photocleavable oligonucleotide tags. After hybridization of probes to slide-mounted FFPE tissue sections, the oligonucleotide tags are released from discrete regions of the tissue via UV exposure. The size and shape of the UV exposure pattern is mutable, allowing for precise investigation into rare or specific cell populations, disease microenvironments, or neural sub-regions. Released tags are quantitated on the standard nCounter® platform, and counts are mapped back to tissue location, yielding a spatially-resolved digital profile of analyte abundance. In this study, we validate a neural cell profiling core panel (~20-plex, RUO) and Alzheimer's pathology and Parkinson's pathology add on modules (~10-plex each, RUO) on the DSP platform. Specific cell population sampling was demonstrated by masking for multiple or individual microglia, neurons, and astrocytes. In addition, diseased human brain tissue was mapped with fluorescent morphology markers highlighting amyloid beta plaques, microglia, and astrocytes to select ROIs for high plex profiling using the validated antibody set. Concentric masks around the plaques (Contour Profiling) allowed for spatially distinct sampling of the amyloid beta plaque microenvironments. The key targets validated include amyloid beta 1-40, amyloid beta 1-42, Tau, Phospho-Tau (S404), ApoE, TMEM119, P2ry12, Park5, Park7, LRRK2, SNCA, GFAP, Iba1, Map-2, Phospho-Tdp-43, Tdp-43, and GFAP. Ongoing efforts will expand the neuro specific antibodies validated for with DSP platform.

Disclosures: **A. Rosenbloom:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **A. Bahrami:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **L. Bogatzki:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **W. Carter:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **J.R. Kuhar:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **K.R. Miller:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **Y. Liang:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **G. Geiss:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc. **J.M. Beechem:** A. Employment/Salary (full or part-time):; Nanostring Technologies, Inc.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.16/AA42

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: The effects ofmethamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA) on hepatocytes

Authors: *N. P. FROMMANN[1], N. HUSSEIN[1], A. TIWARI[1], F. S. HALL[2];
[1]Pharmaceut. Sci., Univ. of Toledo, Toledo, OH; [2]Pharmacol., Univ. of Toledo Col. of Pharm. and Pharmaceut. Sci., Toledo, OH

Abstract: In response to legal restrictions on methamphetamine (MA) and 3,4-methylenedioxymethamphetamine (MDMA), there has been an increase in the use of synthetic psychoactive cathinone's (SPCs). SPCs are structurally similar to MA and MDMA; however, there are some questions as to whether the drugs in this class share similar hyperthermic, lethal, and neurotoxic effects as MA and MDMA. Clinical reports suggest that they produce serious hepatocellular damage, contributing to their serious toxic and lethal effects. In studies in mice and zebrafish we have shown that MA, MDMA and SPCs induce lethal effects that are associated with seizures, perhaps indicative of uncontrolled glutamatergic activation. In a series of experiments, Halpin and Yamamoto (2012, 2013, 2014) have shown that MA induces liver impairments and elevations in plasma ammonia, that contribute to increased glutamatergic activity. In our own studies we have also observed that liver tissue was severely damaged by exposure to these drugs, encouraging the use of liver cells to further understand how these drugs work. In the following project different concentrations of MA and MDMA were used in order to determine the IC50 values and the mechanism of cell death. The obtained IC50 values of MA and MDMA, through MTT assay, were used to define dose ranges for subsequent studies: a concentration which would not cause any cell death (0mM), a concentration which is below our observed IC50 (1mM), a concentration just above the observed IC 50 values (3mM), and a concentration which would almost completely inhibit cell survival (10 mM). In order to determine the mechanism of cell death, this study will include MTT assays, LDH assays, ADP/ATP ratio assays, and Western blots, as well as information obtained through the Incucyte live cell imaging system. MTT assays will provide a concentration of drug which inhibits 50 percent of cell death. LDH assay will be another mechanism to prove that MA and MDMA are killing the cells, as dying cells release lactate dehydrogenase. Western blots will be carried out to help to determine the mechanism of cell death by showing whether apoptotic markers are present at different time points. ATP/ADP ratio assays will provide us with an insight as to whether apoptosis or necrosis is occurring as well. The overall goal here being to determine the mechanism of cell death, first at normal physiological temperature (37°C), and then continue this

process to determine the mechanism of cell death at hyperthermic temperature (40.5°C), mimicking the temperature increases in individuals who consume these drugs.

Disclosures: **N.P. Frommann:** None. **N. Hussein:** None. **A. Tiwari:** None. **F.S. Hall:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.17/AA43

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Viral gene delivery of the transcription factor REST into organotypic hippocampal cultures

Authors: *R. BUTLER-RYAN;
Univ. of Leeds, Leeds, United Kingdom

Abstract: Organotypic slice cultures are a valuable method for studying many neurological disorders, retaining much of the network and architecture of the tissue and providing a more in-vivo-like model than cell line research. However, genetic manipulation of these cultures still remains difficult, with low transfection rates. Here is described for the first time, an effective novel technique for introducing a gene with a GFP tag into organotypic hippocampal cultures using an adenoviral delivery system, with around 40% efficiency rate of cell infection. The cultures are viable up to 1 week following adenoviral infection in vitro, when toxicity begins to cause some cell death. Adenovirus is shown to infect some neurons and all glia. This method is used to deliver the gene for the transcription factor REST (Repressor element 1-silencing transcription factor). REST is a widespread repressor of around 2000 target genes, and is implicated in many neurological disorders, such as ischaemic stroke and epilepsy, Huntington's, Parkinson's and Alzheimer's diseases, and schizophrenia. By using this simple method to manipulate REST in organotypic hippocampal cultures, the physical consequences of altered REST expression and its role in different disease models, can be assessed by various techniques.

Disclosures: **R. Butler-Ryan:** None.

Poster

088. Molecular and Biochemical Techniques

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 088.18/AA44

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: Neuroscience program, Central Michigan University
College of Medicine, Central Michigan University
Department of Chemistry and Biochemistry, Central Michigan University
Field Neurosciences Institute
John G. Kulhavi Professorship in Neuroscience

Title: Delivery of different-sized dendrimers and large plasmids to the brain following intracranial injections in C57BL/6J mice

Authors: *B. SRINAGESHWAR[1,2,3], M. FLORENDO[1,3], A. WEDSTER[1,2], P. OTERO[1,2], C. E. THOMPSON[1,2], S. KONERU[1,2], B. MACDONALD[1,2], R. CRANDALL[1,2], M. M.-M. ANDREWS[1,2,3], N. MUNRO[1,2,3], A. ANTCLIFF[1,2,3], A. AL-GHARAIBEH[1,2], A. FIGACZ[1,3], D. STORY[1,2,4], S. BAIYASI[1,2], D. SWANSON[5], A. SHARMA[5], G. L. DUNBAR[1,2,4,6], J. ROSSIGNOL[1,3,2];
[1]Field Neurosciences Inst. Lab. for Restorative Neurol., [2]Program in Neurosci., [3]Col. of Med., [4]Psychology, [5]Chem. and Biochem., Central Michigan Univ., Mount Pleasant, MI; [6]Field Neurosciences Inst., Saginaw, MI

Abstract: Dendrimers are 3-dimensional nanomolecules that have branches having various biomedical applications. Previous research indicates that the G4 dendrimers having 100% amine surface (G4-NH_2) are highly toxic to cells *in vitro* and *in vivo*, due to their positively charged amine groups on the surface. Therefore, to reduce the toxicity of these dendrimers, we have modified them to have more neutral functional groups, containing only 10% of the surface covered with NH_2 and remaining 90% of the surface covered with hydroxyl groups (-OH; G4-90/10). Our work indicates that these surface-modified dendrimers are taken up by various cultured neurons, glia, and stem cells,) and by brain cells *in vivo*. The toxicity assay shows that these modified dendrimers are less toxic compared to the pure 100% amine surface dendrimers. Our previous and on-going studies also show that the dendrimers can cross the blood-brain barrier (BBB) following carotid- and tail-vein injections. However, prolonged presence of different-sized surface modified dendrimers (G1-90/10 and G4-90/10) were observed up to 3 weeks following unilateral intrastriatal injections in both hemispheres, indicating that these dendrimers migrate via corpus callosum. Furthermore, these dendrimers migrate at different rates, depending on their size (G1 or G4). Moreover, the G4-90/10 dendrimers are capable of forming complex with plasmid DNA of various sizes (up to 11kb) and can deliver them to different stem cells *in vitro* and to the brain cells *in vivo*. We have delivered two non-coding plasmids with reporter genes (6kb and 10kb) *in vitro* to stem cells. As one of our interests lies in using human derived brain-derived neurotrophic factor (hBDNF) as a potential therapy for Huntington's disease (HD), we have also delivered hBDNF (gene coding plasmid) using dendrimers in MSCs and quantified the amount of hBDNF secreted using ELISA following the plasmid delivery. Our research findings show that our (1) dendrimers are taken up by cultured neurons; (2) G1 and G4 dendrimers migrate in the brain following unilateral injections into the striatum for at least 3 weeks and that G1 dendrimers migrate at a faster rate than do the G4 type; and (3) dendrimers can deliver large non-coding plasmids and coding plasmids to the cells *in*

vitro and *in vivo*. The future direction of our research involves systemic delivery of large plasmids using dendrimers *in vivo*.

Disclosures: B. Srinageshwar: None. **M. Florendo:** None. **A. Wedster:** None. **P. Otero:** None. **C.E. Thompson:** None. **S. Koneru:** None. **B. Macdonald:** None. **R. Crandall:** None. **M.M. Andrews:** None. **N. Munro:** None. **A. Antcliff:** None. **A. Al-Gharaibeh:** None. **A. Figacz:** None. **D. Story:** None. **S. Baiyasi:** None. **D. Swanson:** None. **A. Sharma:** None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.01/DP14/BB1

ControlExtraData.DynamicPosterDisplay:
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Topic: I.02. Systems Biology and Bioinformatics

Support: Medical Research Council awards MC_UU_12024/2 and MC_UU_12024/1

Title: CHemoarchitectonic Atlas of the Mouse thalamus as a BNDU opEn Resource (CHAMBER): A publicly-accessible online database of protein expression in mouse brain

Authors: *K. C. NAKAMURA, B. MICKLEM, N. BERRY, G. SPAGNOL, A. SHAROTT, P. J. MAGILL;
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Abstract: Accurate determination of different brain regions — as defined by their specialised inputs, outputs, and constituent cell types — is critical for understanding brain function. The ex vivo use of so-called Nissl staining to reveal the ‘cytoarchitecture’ of the brain has proved to be important for delineating brain regions. However, there are many examples where cytoarchitecture alone cannot disambiguate functionally-distinct brain regions. Empirical delineation of brain regions can be facilitated by studying ‘chemoarchitectonics’, that is, features emerging from distinct patterns of protein expression. Specific antibodies are often used to localise proteins in the brain ex vivo, i.e. with immunofluorescence or immunohistochemistry, followed by tissue imaging. However, many published images of brain tissue are of insufficient resolution and tractability for end users to interrogate them for their own purposes. In summary, there remains significant scope for improving the quality, usability, and accessibility of chemoarchitectonic data for delineating brain regions.
Here, we introduce CHAMBER (CHemoarchitectonic Atlas of the Mouse thalamus as a BNDU opEn Resource), a new chemoarchitectonic atlas of the mouse thalamus and other brain regions

that was made by researchers at the MRC Brain Network Dynamics Unit (MRC BNDU) for use by the wider research community. We acquired high-resolution microscopic images (32,510 x 14,510 pixels with XY pixel size of 0.64 µm, 16-bit per channel) of 33 parasagittal sections of a mouse brain hemisphere that were fluorescently labelled for calretinin, calbindin and parvalbumin immunoreactivities, and Nissl substance. Tiled images were corrected post hoc for transverse chromatic aberrations and 'shadowing' artefacts, and then seamlessly stitched together, and aligned to a published stereotaxic mouse brain atlas. The processed images with structural annotations were made available using an open-source microscopic image managing software (OMERO), offering a 'Google Maps-like experience' for image interrogation. The first phase of CHAMBER is now live and online, and can be readily accessed from the MRC BNDU Data Sharing Platform (https://data.mrc.ox.ac.uk/chamber).

Disclosures: K.C. Nakamura: None. **B. Micklem:** None. **N. Berry:** None. **G. Spagnol:** None. **A. Sharott:** None. **P.J. Magill:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.02/BB2

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant R01MH116176

Title: Enhanced and unified anatomical labeling for a common mouse brain atlas

Authors: *U. CHON[1], D. J. VANSELOW[2], K. C. CHENG[2], Y. KIM[1];
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Abstract: Anatomical atlases in standard coordinates are necessary for the interpretation and integration of research findings in a common spatial context. For mouse, the most commonly used brain atlases are Franklin and Paxinos (FP) atlas based on histological staining of a single mouse brain and the common coordinate framework (CCF) 3D digital atlas from the Allen Institute for Brain Science. However, these atlases have accumulated inconsistencies in anatomical delineations and nomenclature, creating confusion among neuroscientists. To overcome these issues, we adopted the FP labels into the CCF to merge two labels in the single atlas framework. We used cell type specific transgenic mice and an MRI atlas to adjust and further segment our labels. Moreover, new segmentations were added to the dorsal striatum using cortico-striatal connectivity data. We digitized our anatomical labels based on the Allen ontology to facilitate integration of our labels as a neuroinformatics tool, created a web-interface for visualization, and provided tools for comprehensive comparisons between Allen and FP

labels. Our open-source labels provide an neuroinformatics platform for future neuroanatomical studies and signify a key step towards a unified mouse brain atlas.

Disclosures: **U. Chon:** None. **D.J. Vanselow:** None. **K.C. Cheng:** None. **Y. Kim:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.03/BB3

Topic: I.02. Systems Biology and Bioinformatics

Support: NRF Korea Grant 2N54930
KIST Grant 2E29180

Title: Comprehensive convergence map of the subthalamic nucleus using connectivity and molecular profiling datasets

Authors: *H. JEON[1], H. LEE[1,2], J. KIM[1,2], L. FENG[1], J. KIM[1,2];
[1]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [2]Div. of Bio-Medical Sci. & Technol., Korea Univ. of Sci. and Technol. (UST), Seoul, Korea, Republic of

Abstract: Since the huge clinical success of the subthalamic nucleus (STN) deep brain stimulation (DBS) in the treatment of Parkinson's disease (PD), there have been extensive studies in trying to identify its structural and functional characteristics and to understand the circuit-level mechanisms of STN-DBS. Recent advances in neuroimaging techniques enabled us to acquire extensive biological knowledges about the STN. However, despite the collaborative efforts from researchers in developing a standardized reference model, much of these new findings remain fragmented and the comprehensive analysis based upon multi-modal data set is still incomplete. This work aims to construct a comprehensive multi-modal map of STN characterized by 1) differential cellular and neurotransmitter receptor distribution on top of 2) detailed topography of major inputs constructed using multi-scale connectivity data from different datasets. Specifically, we constructed comprehensive input connectivity map of STN via joint modelling of our indirect inhibitory pallido-subthlamic connectome data using antero- and retro-grade viral tracers and mammalian GFP reconstitution across synaptic partner (mGRASP), with hyperdirect cortico-subthalamic connectome described in publicly available dataset such as Allen Mouse Brain (http://connectivity.brain-map.org) and MouseLight (http://mouselight.janelia.org). Then, the spatial distribution of molecular and cellular profile was analyzed in context of input connectivity topography using kernel canonical correlation analysis (kCCA). Based on the agreements and discrepancies between multi-modal structural and molecular maps, we identified a convergent topological organization of major input connectivity

and neurochemical profile within STN, suggesting the existence of functional layer far more intricate than the conventionally posited discrete tripartite STN subdivisions. Taken together, our comprehensive anatomical and molecular map of STN will deepen our understanding of network underlying cortical and pallidal connections within STN and may shed light on mechanisms of STN-DBS.

Disclosures: H. Jeon: None. **H. Lee:** None. **J. Kim:** None. **L. Feng:** None. **J. Kim:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.04/BB4

Topic: I.02. Systems Biology and Bioinformatics

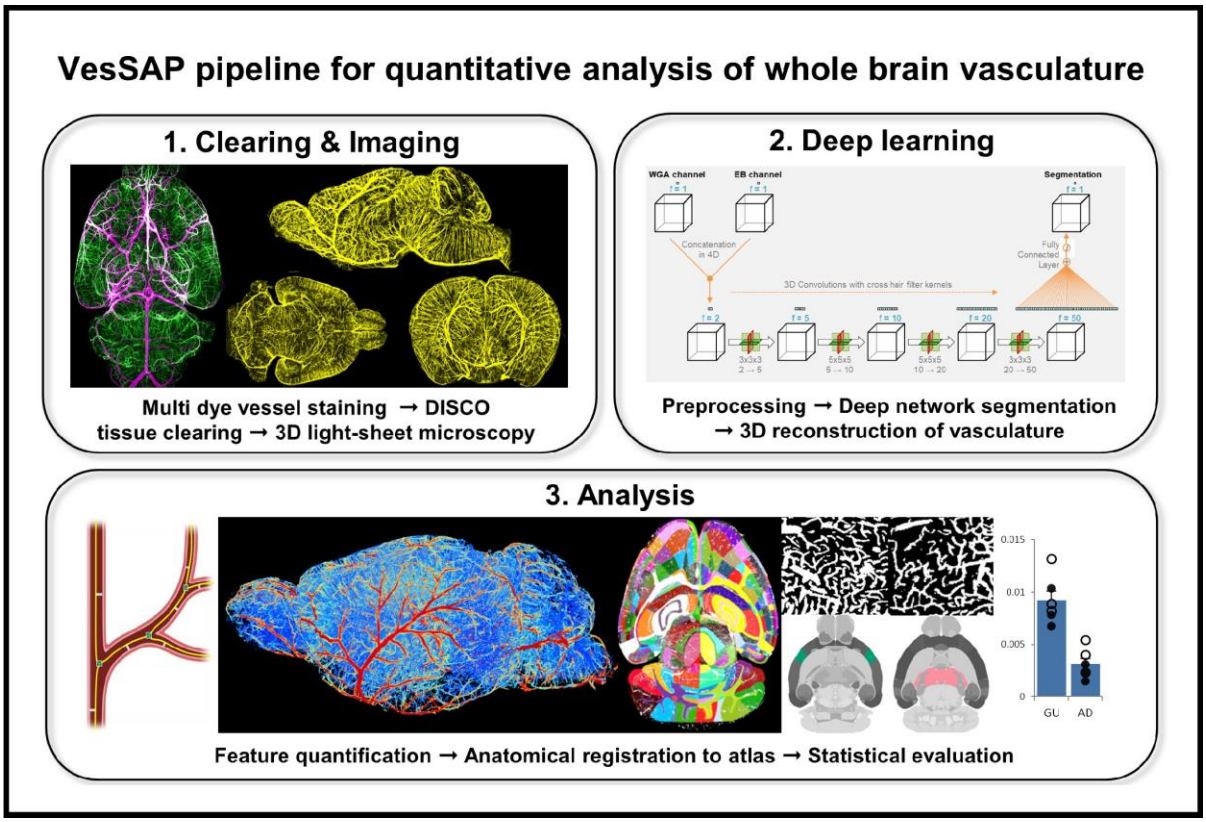
Support: ERA-Net Neuron (01EW1501A to A.E.)
Fritz Thyssen Stiftung (A.E., Ref. 10.17.1.019MN)
DFG (A.E., Ref. ER 810/2-1), NIH (A.E.)
Helmholtz ICEMED Alliance (A.E.)
German Federal Ministry of Education and Research via the Software Campus initiative (O.S.)
GPU Grant Program

Title: Automated analysis of whole brain vasculature using machine learning

Authors: *M. I. TODOROV[1,2,3], J. C. PAETZOLD[4,5], O. SCHOPPE[4], G. TETTEH[4], V. EFREMOV[4], K. VÖLGYI[3], M. DÜRING[3,6], M. DICHGANS[3,7,6], M. PIRAUD[4], B. MENZE[4,5], A. ERTÜRK[3,6];
[1]Klinikum der Univ. München, Munich, Germany; [2]Grad. Sch. of Systemic Neurosciences, Munich, Germany; [3]Inst. for Stroke and Dementia Research, Univ. Hospital, Ludwig-Maximilians-Universität LMU, Munich, Germany; [4]Dept. of Computer Science, Tech. Univ. of Munich, Munich, Germany; [5]Munich Sch. of Bioengineering, Tech. Univ. of Munich, Munich, Germany; [6]Munich Cluster for Systems Neurol. (SyNergy), Munich, Germany; [7]German Ctr. for Neurodegenerative Dis. (DZNE), Munich, Germany

Abstract: Tissue clearing methods enable imaging of intact biological specimens without sectioning. However, reliable and scalable analysis of such large imaging data in 3D remains a challenge. Towards this goal, we developed a deep learning-based framework to quantify and analyze the brain vasculature, named Vessel Segmentation & Analysis Pipeline (VesSAP). Our pipeline uses a fully convolutional network with a transfer learning approach for segmentation. We systematically analyzed vascular features of the whole brains including their length, bifurcation points and radius at the micrometer scale by registering them to the Allen mouse

brain atlas. We reported the first evidence of secondary intracranial collateral vascularization in CD1-Elite mice and found reduced vascularization in the brainstem as compared to the cerebrum. VesSAP thus enables unbiased and scalable quantifications for the angioarchitecture of the cleared intact mouse brain and yields new biological insights related to the vascular brain function (Todorov & Paetzold *et al.* 2019).
Reference: Todorov MI, Paetzold JC, Schoppe O, Tetteh G, Efremov V, Völgyi K, Düring M, Dichgans M, Piraud M, Menze B, Ertürk A. Automated analysis of whole brain vasculature using machine learning. BioRxiv: https://www.biorxiv.org/content/10.1101/613257v1



Disclosures: **M.I. Todorov:** None. **J.C. Paetzold:** None. **O. Schoppe:** None. **G. Tetteh:** None. **V. Efremov:** None. **K. Völgyi:** None. **M. Düring:** None. **M. Dichgans:** None. **M. Piraud:** None. **B. Menze:** None. **A. Ertürk:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.05/BB5

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant R01EY020560
NIH Grant K99EY027844

Title: Ascot identifies key regulators of neuronal subtype-specific splicing

Authors: *J. P. LING[1], C. WILKS[2], B. LANGMEAD[2], S. BLACKSHAW[1];
[1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Public archives of next-generation sequencing data are growing exponentially, but the difficulty of marshaling this data has led to its underutilization by scientists. Here we present ASCOT, a resource that allows researchers to summarize, visualize, and query alternative splicing patterns in public RNA-Seq data. ASCOT enables rapid identification of splice-variants across tens of thousands of bulk and single-cell RNA-Seq datasets in human and mouse. To demonstrate the utility of ASCOT, we first focused on the nervous system and identified many alternative exons used only by a single neuronal subtype. We then leveraged datasets from the ENCODE and GTEx consortiums to study the unique splicing patterns of rod photoreceptors and found that *PTBP1* knockdown combined with overexpression of *MSI1* and *PCBP2* activates rod-specific exons in HepG2 liver cancer cells. Furthermore, we observed that *MSI1* targets intronic UAG motifs proximal to the 5' splice site and interacts synergistically with *PTBP1* downregulation. Finally, we show that knockdown of *MSI1* in the retina abolishes rod-specific splicing. This work demonstrates how large-scale analysis of public RNA-Seq datasets can yield key insights into cell type-specific control of RNA splicing and underscores the importance of considering both annotated and unannotated splicing events. ASCOT splicing and gene expression data tables, software, and interactive browser are available at http://ascot.cs.jhu.edu.

Disclosures: J.P. Ling: None. **C. Wilks:** None. **B. Langmead:** None. **S. Blackshaw:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.06/BB6

Topic: I.02. Systems Biology and Bioinformatics

Title: Coupled autoencoders for multimodal cell type analysis

Authors: *R. GALA, N. GOUWENS, A. BUDZILLO, Z. YAO, F. BAFTIZADEH, O. PENN, B. TASIC, G. MURPHY, H. ZENG, U. SÜMBÜL;
Allen Inst., Seattle, WA

Abstract: Understanding the diversity of neuronal cell types is a fundamental goal of neuroscience. Single-cell RNA sequencing is a high throughput method to capture transcriptomic profiles of neurons. The degree to which such molecular profiles overlap with other functionally

relevant properties of neurons such as electrophysiology and anatomy is not clear. Patch-seq experiments enable multimodal profiling of individual neurons. Here, we describe a computational method to quantify correspondence between different data modalities and apply it to a Patch-seq dataset consisting of > 1500 mouse visual cortex neurons for which both, transcriptomic and electrophysiological, profiles were obtained.
Our method uses multiple, coupled instances of deep neural networks called autoencoders. The individual autoencoders learn to compress data from a single modality into a low dimensional space. Mismatches between the low dimensional representations being learned simultaneously by the different coupled autoencoders are penalized as part of the network training procedure. Thus the coupled autoencoders attempt to learn low dimensional representations that not only encode the input modality faithfully, but also maintain consistency across the different data modalities. Our analysis of the Patch-seq dataset reveals a surprisingly high correspondence between transcriptomic and electrophysiological features of neurons. We show that representations obtained by coupled autoencoders can (i) be used to identify consistent cell types based on multimodal data, and (ii) predict cross modal data accurately. Our results are among the first to quantify the extent to which neuron type identity is preserved across disparate data modalities.

Disclosures: **R. Gala:** None. **N. Gouwens:** None. **A. Budzillo:** None. **Z. Yao:** None. **F. Baftizadeh:** None. **O. Penn:** None. **B. Tasic:** None. **G. Murphy:** None. **H. Zeng:** None. **U. Sümbül:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.07/BB7

Topic: I.02. Systems Biology and Bioinformatics

Title: Computational comparison of glycopeptides derived from rabies virus used as a vector in structural properties and predicted targets

Authors: *A. E. GONZALEZ-SANTIAGO[1], **R. CASTAÑEDA-ARELLANO**[1], M. G. SANCHEZ-PARADA[1], I. G. AGUILAR-GARCIA[2], R. D. CASTRO-TORRES[3], A. A. SOBREVILLA-NAVARRO[1];
[1]Biomed. Sci., Univ. de Guadalajara, Centro Universitario de Tonala, Mexico; [2]Neurociences Dept., Univ. de Guadalajara, Centro Universitario de Ciencias de la Salud, Mexico; [3]Inst. de Neurociencies, Univ. de Barcelona, Barcelona, Spain

Abstract: Drug bioavailability in the central nervous system (CNS) still remains a challenge. It is necessary to develop new internalization mechanisms with both effective receivers and transporters. It is possible to take advantage of the structure of some viruses, such as rabies, to

facilitate the entry and transport of substances into the CNS. Glycoproteins derived from the rabies virus (RVG)-modified have been proposed to improve the administration of drugs in neurodegenerative diseases. In this work, we propose to characterize rabies peptides glycoproteins used in the literature as vectors, with bioinformatic tools including BIOPEP from University of Warmia and Mazury in Olsztyn, SwissTargetPrediction from Swiss Institute of Bioinformatics, DAVID Bioinformatics Resources 6.8 from Laboratory of Human Retrovirology and Immunoinformatics (LHRI) and KEGG Pathways developed by Kanehisa Laboratories. The structure properties, protein interactions, prediction of molecular targets class and target cellular pathways of 7 designs of rabies peptides are analyzed and their variability is compared based on a combination of 2D and 3D similarity measures with known ligands with a probability for the query molecule assumed as bioactive. Target classes as Family A G protein-coupled receptor, membrane receptors, surface antigens, kinases, and secreted proteins where identified as predominant predicted targets

Disclosures: **A.E. Gonzalez-Santiago:** None. **R. Castañeda-Arellano:** None. **M.G. Sanchez-Parada:** None. **I.G. Aguilar-Garcia:** None. **R.D. Castro-Torres:** None. **A.A. Sobrevilla-Navarro:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.08/BB8

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH R24MH114799

Title: Generative and discriminative approaches for neuronal cell type

Authors: M. CALABRESI, *M. VILLAFANE-DELGADO, W. GRAY-RONCAL;
Johns Hopkins Univ. Applied Physics Laborator, Laurel, MD

Abstract: We present approaches to generate and classify neurons according to cell type given skeletonized reconstructions. For the generation component, we draw from the Linguistics and Computer Science domains to apply Probabilistic Context-Free Grammars (PCFGs), trained on skeletonized cells from the NeuroMorpho database and the Allen Brain Atlas. A PCFG models branching trees by generating child nodes from parent nodes according to transition rules. Each transition rule considers only the current node and a probability value. Our PCFGs generate trees with nodes whose features include samples from joint probability distributions in order to simulate both the branching structure of a cell and place it concretely in 3-space. The resultant cell models are visually distinguishable from real skeletons, but this step represents progress in the work of modelling the branching structure with respect to cell type, and creates a syntax and

associated transition probabilities to better understand and leverage the underlying structure. For the discriminative approach, we utilize a Random Forest Algorithm to classify cells by type. The algorithm considers features for seven different cell types: interneuron, pyramidal, Purkinje, Kenyon, stellate, astrocyte, bipolar types. When all seven types are compared simultaneously, the classifier is able to identify them with above 80% accuracy. When the classifier is tasked only with distinguishing between two types of cells, it achieves improved performance, often achieving perfect classification (depending on the pair of cell types). The features used in the classification include standard neuroscience measurements (e.g., branch angle, arborization length) and others developed or applied in the context of this project (e.g., distance from farthest leaf node to its root, a balance metric relating graph center of mass to midpoint). We are currently exploring extensions of this work to classify cell types when only portions of a skeleton are observed (such as when neuron arbors are reconstructed using automated methods).

Disclosures: M. Calabresi: None. **M. Villafane-Delgado:** None. **W. Gray-Roncal:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.09/BB9

Topic: I.02. Systems Biology and Bioinformatics

Support: JHU/APL Internal Research and Development

Title: Training a neural model using the *C. elegans* connectome to perform exploration tasks

Authors: *R. NORMAN-TENAZAS[1], R. S. RAIS[2], E. C. JOHNSON[1], W. GRAY-RONCAL[1]; [1]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Understanding the link between information processing in neural circuits and behavior remains a key goal in neuroscience. Network circuits extracted from the brain can be represented as a graph, where neurons are nodes and synapses are edges; attributes such as edge weights can provide additional context and information about the flow of information through the network. With the addition of dynamic neuronal models, these connectomes can be modeled as a system that takes sensory information as inputs and produces outputs that act on the surrounding environment, process sensory input and produce output. Simulating this dynamic model can help interpret experimental results and aid hypothesis development. Simulation can be done using a variety of tools with various levels of fidelity and objective functions (e.g., performance, low-level biological fidelity, concurrence with neuroscience theory). Given the extreme simplicity of the *C. elegans* nematode and its complete connectome, it is an optimal candidate for initial research discovery. Using simple neuron models, its entire nervous system can be readily simulated and we have demonstrated this on low-level hardware.

In a model of *C. elegans* running in a python simulation environment, we investigate the exploration behavior of *C. elegans* by modifying the weights of a *C. elegans* connectome with a genetic algorithm. Therefore, we have investigated training the connectome to perform simple tasks in simulation by using a simple integrate-and-fire neuron model and a simple kinematic model of an agent. Example environments include gradient following, finding food, avoiding collisions, and noxious stimuli. We study our trained connectome in changing environmental conditions and investigate the effects of ablating neurotransmitter pathways on behavior. This work has implications for low-complexity, bio-inspired robotic exploration algorithms which may be more robust than reinforcement learning methods using artificial neural networks.

Disclosures: **R. Norman-Tenazas:** A. Employment/Salary (full or part-time):; Johns Hopkins Applied Physics Laboratory. **R.S. Rais:** None. **E.C. Johnson:** A. Employment/Salary (full or part-time):; Johns Hopkins Applied Physics Laboratory. **W. Gray-Roncal:** A. Employment/Salary (full or part-time):; Johns Hopkins Applied Physics Laboratory.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.10/DP12/BB10

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant 1R24MH114785-01A1
IARPA MICrONS Program

Title: bossDB: A scalable cloud-based data ecosystem for storage and sharing of electron microscopy and x-ray microtomography datasets

Authors: S. HIDER, D. PRYOR, T. GION, L. RODRIGUEZ, C. FERNANDES, C. BISHOP, J. MATELSKY, M. WILT, J. DOWNS, W. GRAY-RONCAL, ***B. A. WESTER**;
Johns Hopkins Univ. APL, Laurel, MD

Abstract: With the advent of new imaging technologies and capabilities, volumetric neuroimaging datasets continue to increase in extent and resolution, creating challenges for data storage, processing, and analysis. Current approaches for storing and processing these datasets are limited in scalability and reliability, especially when considering performance, cost-efficiency, and secure, distributed access. This is especially impactful as data sharing increases in importance across numerous neuroscientific communities that contain specialized data generators, data processors, and data analyzers. As image volumes routinely reach terabyte and petabyte scale, automated processing pipelines will replace manual inspection and annotation,

and will require a data storage service and ecosystem with scalable endpoints, data access services, caching, and compute capabilities.
The Block and Object Storage Service (bossDB), a scalable cloud-native data ecosystem for volumetric data, was developed to address each of these challenges, to streamline how users store and share data, and enable community-developed analytics workflows by facilitating large-scale batch processing (e.g. SABER), annotation and metadata storage, and web-based visualization (e.g. neuroglancer). It supports multidimensional and volumetric neuroscience datasets, including those generated by electron microscopy and X-ray microtomography techniques. A well-documented interface supports a suite of tools to allow a user to easily ingest, validate, visualize, and query neuroscience data, making it possible for scientists to discover and share insights on massive datasets. The bossDB ecosystem is open source, and a robust toolkit is provided to enable additional development and integration with community and user-developed data resources and tools, as well as the ability for a research team to stand up their own bossDB stack.
bossDB platform instances are currently supporting over 20 geographically distributed academic partners in the neuroscience community, including data collected with various imaging and time-series modalities. The bossDB system is also currently storing a neuroanatomical dataset that exceeds 1PB in size.

Disclosures: **S. Hider:** None. **D. Pryor:** None. **T. Gion:** None. **L. Rodriguez:** None. **C. Fernandes:** None. **C. Bishop:** None. **J. Matelsky:** None. **M. Wilt:** None. **J. Downs:** None. **W. Gray-Roncal:** None. **B.A. Wester:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.11/BB11

Topic: I.02. Systems Biology and Bioinformatics

Support: R24MH114799

Title: Transfer learning analysis of computer vision tools for automated analysis of connectivity in electron microscopy data

Authors: ***E. C. JOHNSON**[1], L. M. RODRIGUEZ[1], R. NORMAN-TENAZAS[1], E. L. DYER[2], W. R. GRAY RONCAL[3];
[1]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; [2]Georgia Inst. of Technol., Atlanta, GA; [3]Applied Physics Lab., Johns Hopkins Univ., Laurel, MD

Abstract: Using Electron Microscopy (EM) imaging techniques, researchers are producing ever larger datasets of structural neuroimaging data at nanoscale resolution. These petascale datasets

offer unprecedented insights into the structure of individual neurons and connectivity patterns at the synapse level, which may give new insights into the connectivity patterns of neurodegenerative disease and the fundamental mechanisms of information processing in cortex. As these datasets continue to be collected, a key bottleneck remains the analysis pipeline which segments neurons, synapses, and subcellular structures out of the raw imaging data. Despite the advances of tools such as Flood Filling Networks, large amounts of painstakingly labeled ground truth data and long training times are required to achieve accurate results. To lower the barrier of entry for investigators collecting new datasets, transfer learning strategies must be developed to rapidly adapt existing, trained models to new datasets while maintaining performance. During transfer learning, algorithms are trained using a labeled, reference dataset before being applied to a new dataset with limited ground truth data. When applied to newly collected EM data, a drop in performance is expected and can be mitigated by fine-tuning the model or transforming the input data to account for changing image statistics. We investigate transfer from a reference EM dataset to a new EM dataset 1) collected in a new region of the nervous system, 2) collected in a different animal model, and 3) collected with a different imaging technology. Data include images from the nervous system of *Drosophila melanogaster* and mouse cortex. We characterize the change in performance of tools for synapse detection and neuron segmentation, including common neural network tools used for segmentation and synapse detection. This study aims to highlight the limitations in transfer to new datasets and steps that should be taken to accelerate segmentation of new imaging data.

Disclosures: E.C. Johnson: None. **L.M. Rodriguez:** None. **R. Norman-Tenazas:** None. **E.L. Dyer:** None. **W.R. Gray Roncal:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.12/BB12

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant R24MH114799

Title: Navigate: Neuroanatomical validation of MR based connectomes

Authors: *H. P. COWLEY, M. VILLAFANE-DELGADO, J. MATELSKY, A. SIVAKUMAR, M. WOLMETZ, W. R. GRAY RONCAL;
The Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: Diffusion-weighted Magnetic Resonance Imaging (DWI) is a powerful tool for imaging the white matter tracts of the human brain and generating an estimate of the macroscale structural connectome. Researchers are investigating the potential utility of these networks for

understanding, diagnosing, and monitoring neurological function and dysfunction. Exploring the biofidelity of these graphs and leveraging multi-channel information to create an improved consensus connectome will facilitate the comparison of healthy and patient populations. Graph-based analyses of DWI generate estimates of putative axonal streamlines; after leveraging volumetric parcellations to convert streamlines to graphs, graph analytic approaches yield insights into white matter connectivity and variability. Many research efforts have focused on assessing and improving the reliability of graph estimation methods, but questions remain about how well these networks represent the true macroscale connectivity of the human brain.
Mean connectomes may be created by averaging registered graphs from a population of subjects to create average measures of connectivity between pairs of regions. To create a robust consensus connectome, we first utilize the open-source pipeline *ndmg* to generate graphs from Human Connectome Project anatomical data. We combined the resulting mean graphs to two additional information sources: established white-matter connectivity tracts and patterns of edge heritability. The fusion of information across these three sources allows us to produce a consensus connectome that better characterizes macroscale connectivity than the signal available from a single, noisy source. Finally, we illustrate a possible application of our approach by using the resulting connectome to constrain features on an example subject classification task. Our framework can be extended to incorporate new information as new data and methods become available (e.g., higher quality connectomes, additional graph constraints). This will enable researchers to compare their generated connectomes to a robust, trusted reference source toward improved approaches for understanding neurological diseases and conditions.

Disclosures: **H.P. Cowley:** None. **M. Villafane-Delgado:** None. **J. Matelsky:** None. **A. Sivakumar:** None. **M. Wolmetz:** None. **W.R. Gray Roncal:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.13/BB13

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH R24MH114799
NIH R24MH114785
IARPA Contract No. 2017-17032700004-005 under the MICrONS program

Title: Rapid and scalable connectome reconstruction assessment through graph analytics

Authors: *J. DOWNS, M. VILLAFAÑE-DELGADO, E. REILLY, M. HUGHES, E. C. JOHNSON, W. GRAY RONCAL;
Res. and Exploratory Develop., Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: Despite a growing interest in nanoscale brain imagery, it is challenging to accurately estimate cortical circuitry from electron microscopy (EM) volumes and assess its quality. A critical component of connectomics analysis is the processing framework which begins with EM images and ultimately yields a graph representation of the neural circuit, where neurons are represented by nodes and synapses by edges. Although humans can often accurately reconstruct these graphs through manual annotation, this process is extremely cost and time intensive, and difficult to scale as imaging volumes increase in size. In the largest petascale datasets, even targeted sparse human annotations or manually proofread circuits are difficult to achieve at a sufficient scale. Therefore, automatic segmentation pipelines are becoming increasingly necessary to process the EM data at scale, resulting in rapid reconstructions and the capability to interrogate the brain at a scale that was unimaginable only a few years ago. However, these automatic pipelines are imperfect, and the resulting graph contains anomalies such as the splits and merges of neurons, which lead to errors in the final graph representation.
To facilitate the use of these errorful, imperfect connectomes, it is important to characterize the accuracy of the derived graph. We have created a suite of tools integrated with a graph database to abstract many of the computer science and infrastructure challenges associated with performing these analyses. Within this framework, we have created tools to produce subgraphs and summary statistics, (e.g., quantifying number of nodes, edges, orphans, loops and connected components). We relate these metrics to neuroscience-informed priors (e.g., number of neurons, synapses, isolates, autapses, and connected regions), and can provide insight into our graph reconstruction through literature references and comparative connectomics studies. With these summary statistics, we can infer when anomalies are present, provide a quality assessment, and identify subgraphs or regions for further inspection.
We can rapidly deploy a graph database locally or in the cloud, and compute summary statistics for a brain graph, providing an easy-to-use capability for connectomics labs. Our tools are able to analyze reconstructed graphs representing hundreds of millions of synapses, and empower us to provide constructive feedback to automatic segmentation algorithms and downstream primary and secondary data science applications.

Disclosures: **J. Downs:** None. **M. Villafañe-Delgado:** None. **E. Reilly:** None. **M. Hughes:** None. **E.C. Johnson:** None. **W. Gray Roncal:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.14/BB14

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH R24MH114799
JHU/APL Internal Research and Development

Title: SMART: Statistical method for annotation reconstruction from traces

Authors: S. MATSON[1], E. P. REILLY[1], M. HUGHES[1], M. WILT[1], C. A. BISHOP[2], E. C. JOHNSON[1], *W. R. GRAY-RONCAL[1];
[1]Applied Physics Lab., Johns Hopkins Univ., Laurel, MD; [2]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: Many challenges in computational neuroscience, biomedical imaging and other domains require rapid, reliable, and robust assessment of path reconstruction, in diverse fields including bronchi segmentation, vasculature mapping, and robotic decision-making. Here, we propose a generalized solution for creating and assessing ground truth paths by leveraging crowd sourcing and decision fusion, and then illustrate the impact in a nanoscale connectomics use case. In connectomics, gold-standard brain graphs are created from expert annotations of electron microscopy images; annotators aim to reconstruct individual neurons and their synapses, which provide connections between these cells. As the scientific community continues to collect larger volumes of neuroimaging data, relying on expert annotators is rapidly becoming infeasible. The fusion method presented in this paper, called Statistical Method for Annotation Reconstruction from Traces (SMART), combines multiple errorful annotations of the same brain region to produce an improved estimate of ground truth. We use Bayesian decision theory and priors based on predicted graph density and annotation quality to determine the likelihood of edge existence. Then, we combine theory and experimental results to determine a threshold for including edges in the reconstructed graph. SMART is flexible and does not assume uniformity of annotation quality. If a more expert annotator (or algorithm) is present, their contribution will outweigh non-experts because the learned priors play a significant role in the fusion process. The SMART method can be easily applied to other use cases, as well as downstream post-processing based on computed edge confidence. The results can be used in many different applications such as training ML algorithms, improving trust in non-expert annotations, and directly for scientific analysis. We validate results against a variety of simulated and experimental data, and find that the proposed methods allow us to create graphs that replicate expert annotations at high levels of sensitivity and specificity while being resource efficient and limiting the need for expert input.

Disclosures: S. Matson: None. **E.P. Reilly:** None. **M. Hughes:** None. **M. Wilt:** None. **C.A. Bishop:** None. **E.C. Johnson:** None. **W.R. Gray-Roncal:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 089.15/BB15

Topic: I.02. Systems Biology and Bioinformatics

Support: JHU/APL Internal Research and Development

Title: A method for repeated neural circuit identification in noisy brain graph data

Authors: *E. P. REILLY[1], M. V. SCHUYLER[2], **J. K. MATELSKY**[1], W. R. GRAY-RONCAL[1];
[1]Intelligent Systems Ctr., [2]The Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: As larger neural circuit data becomes broadly available to the research community, researchers look to the brain to understand why humans perform certain tasks robustly and efficiently and the underlying circuitry for some neurological disorders. In particular, discovery of repeated structure in large, newly collected brain image volumes would support the conjecture that the brain is modularly organized. At the same time, information extracted from brain imaging is inherently noisy due to errors manifested at all stages of the reconstruction process and the inability of humans to proofread or ground truth the vast amount of data available. Robust methods to analyze brain data could lead to the discovery of repeated brain structure, even in the presence of errors.
We define a probabilistic approach to identify significant subgraph structures within imperfect graph data, allowing us to capture uncertainty in our discovery process and perform inference over noisy data. Our probabilistic approach uses graph data where edges are not binary, but rather have some confidence level, or weight, associated with them, as you might expect to obtain from a computer vision algorithm. While current methods often threshold the edges based on their weights, we instead use the edge weights to define a random graph model similar to an Erdös-Rényi model, but where the edges have varying probabilities based on the provided edge weight, thus creating a data-driven probabilistic graph. The intuition is that the true, underlying graph and small variations of it would occur with high probability in this model. Once the random graph model is defined, we use standard probabilistic graph techniques and sampling to determine the distribution of a subgraph occurring in this data-driven model. This distribution may then be compared with that from a standard Erdös-Rényi model with similar expected density using the Kolmogorov-Smirnov test. In other words, we compare the existence of a subgraph in our data-driven random graph model with the existence of that subgraph in a purely random model. Thus, we work towards identifying structural motifs in the presence of unknown reconstruction errors. We apply our methods to a handful of small subgraphs for initial testing of this approach and compare to the results obtained using a thresholded graph.

Disclosures: E.P. Reilly: None. **M.V. Schuyler:** None. **J.K. Matelsky:** None. **W.R. Gray-Roncal:** None.

Poster

089. Connectomics Analytics I

Location: Hall A

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Topic: I.07. Data Analysis and Statistics

Support: NIH/NIBIB (P41-EB018783)
NIH/NIBIB (R01-EB026439)
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NIH/NICHD (R25-HD088157)
NIH/NIMH (P50-MH109429)
US Army Research Office (W911NF-14-1-0440)

Title: Passive functional mapping of eloquent cortex using stereo-electroencephalography (SEEG)

Authors: *A. VATO[1,2], G. LI[3,1], S. JIANG[4], D. ZHANG[3], L. CHEN[4], N. RAVIV[5], G. SCHALK[1,6,7];
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Abstract: Intraoperative electrocortical stimulation (ECS) is the gold standard for mapping eloquent cortex prior to resective neurosurgery for tumors or seizure foci. While many studies have shown that ECS is useful for minimizing postsurgical deficits, it has several limitations that include the long duration of the procedure (several hours), and it can cause epileptic seizures. Over the past decade, a number of studies have described an alternative functional mapping method that passively records electrocorticographic (ECoG) signals using grid or strip electrodes that are placed directly on the surface of the brain. This technique measures ECoG signals in the broadband gamma frequency range (70-170 Hz), which are known to be reflective of local cortical processing and determines those locations whose broadband gamma activity changes with the task. Studies have described the application of this passive technique to map motor, sensory, or language function. Stereo-encephalography (SEEG) is a different intracranial recording technique that uses depth electrodes inserted in the brain to explore subcortical regions. Because SEEG is better tolerated by the patients compared to a full craniotomy, it has been increasingly adopted in centers worldwide. SEEG electrodes are primarily placed to identify seizure foci rather than for functional mapping. At the same time, once implanted, identifying functional cortical locations or white matter tracts would still be useful, but no previous study applied passive functional mapping to data acquired using the SEEG technique. In this study, we demonstrate the feasibility of passive functional mapping of eloquent cortex using stereo-electroencephalographic recordings. We evaluated data from 24 right-handed patients with intractable epilepsy who were implanted with SEEG electrodes for pre-surgical assessment of a seizure focus. Signals were collected at the bedside of the patients from 257 implanted electrode shafts (3393 contacts in total) while they were performing different types of hand or arm movements. Our results confirm that passive functional mapping readily identifies cortical and subcortical locations related to hand/arm sensorimotor function. Across all 24

patients, the topography of these locations is similar to those identified previously using subdurally-placed ECoG electrodes. The results of our study demonstrate that passive functional mapping techniques can readily be applied in clinical practice to patients who have been implanted with SEEG electrodes to derive functional maps. This additional information should reduce postoperative deficits.

Disclosures: **A. Vato:** None. **G. Li:** None. **G. Schalk:** None. **N. Raviv:** None. **D. Zhang:** None. **S. Jiang:** None. **L. Chen:** None.

Poster

089. Connectomics Analytics I

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NIH/NIMH (P50-MH109429)
US Army Research Office (W911NF-14-1-0440)

Title: Localization of the central sulcus and hand area sensorimotor cortex using electrocorticographic activity evoked by median nerve stimulation

Authors: ***T. XIE**[1,2], Z. WU[3], A. VATO[1,4], Q. GUO[3], H. YE[2], X. SHENG[2], X. ZHU[2], G. SCHALK[1,5,6], L. CHEN[3];
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Abstract: Accurate identification of functional regions in the cortex is essential during resective neurosurgery for tumors, seizure foci, and vascular malformations. A standard intraoperative procedure for localizing an important landmark such the central sulcus consists of visually identifying the polarity of the N20/P20 component of somatosensory potentials evoked by median nerve stimulation and recorded using electrocorticographic (ECoG) electrodes placed on the surface of the brain. After the localization of the central sulcus, functional mapping of sensorimotor regions can be obtained by using electrocortical stimulation (ECS) of the cortex.

However, the visual inspection of evoked potentials is time consuming and depends on expert understanding, and ECS is very time consuming and can introduce seizures. Here we introduce a new method based on quantitative analyses of ECoG-based evoked potentials resulting from intraoperative median nerve stimulation. This technique automatically localizes the central sulcus and obtains a functional map of hand areas of sensorimotor cortex innervated by the median nerve. We evaluated eight patients who underwent an awake craniotomy and median nerve stimulation for the purpose of localizing the central sulcus prior to tumor resection. As additional control, we also applied vibrotactile stimulation to the tip of the index finger. Our method defined the central sulcus by using the N20/P20 to automatically classify a location into either the frontal lobe or parietal lobe, and hand area sensorimotor cortex as those locations whose activity in the broadband gamma frequency range (60-140 Hz) changed with the median nerve stimulation. When compared to results made by a neurosurgeon, we found that our method to identify the central sulcus had a high sensitivity (96.5%) and high specificity (93.1%). Furthermore, the functional maps resulting from median nerve stimulation and vibrotactile stimulation were substantially congruent as well (84.6% sensitivity and 72.6% specificity). With further validation, the proposed automated method could provide a new tool for invasive neurosurgery to automatically identify the central sulcus and hand motor regions in just a few minutes.

Disclosures: **T. Xie:** None. **Z. Wu:** None. **A. Vato:** None. **Q. Guo:** None. **H. Ye:** None. **X. Sheng:** None. **X. Zhu:** None. **G. Schalk:** None. **L. Chen:** None.

Poster

089. Connectomics Analytics I

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Deustche Forschungsgemeinschafts (DFG, German Research Foundation), 122679504 – SFB 874

Title: Universal SpikeDeeptector: A deep-learning based method for detecting neural spiking activity of different species

Authors: *M. SAIF-UR-REHMAN[1,2], R. LIENKAEMPER[1,3], S. DYCK[1,3], A. RAYAN[4], Y. PARPALEY[1], J. WELLMER[1], C. LIU[7], B. LEE[7], S. KELLIS[8], D. MANAHAN-VAUGHAN[4], O. GÜNTÜRKÜN[5], R. A. ANDERSEN[8], I. IOSSIFIDIS[9], T. GLASMACHERS[6], C. KLAES[1,3]; [1]Dept. of Neurosurg., [2]Fac. of Electrical Engin. and Information Engin., [3]Intl. Grad. Sch. of Neurosci., [4]Dept. of Neurophysiol., [5]Inst. of Cognitive Neurosci., [6]Inst. of Neuroinformatics,

Ruhr Univ., Bochum, Germany; [7]Neurorestoration Ctr., USC, Los Angeles, CA; [8]Biol. Div., Caltech, Pasadena, CA; [9]Inst. of Neuroinformatics, HRW Univ. of Applied Sci., Bottrop, Germany

Abstract: State-of-the-art microelectrode array technology enables simultaneous, large-scale single unit recordings from hundreds of channels. Identification of channels recording neural data as compared to noise is the first step for all further analyses. Automatizing this process aims at minimizing the human involvement and time for manual curation. In our previous study, we introduced the "SpikeDeeptector" (SD), which enables us to automatically detect and track channels containing neural data from different human patients implanted with different types of microelectrodes across different brain areas. SD works on human data and to some extent on the data of non-human primates (NHPs). However, to make SD more versatile we proposed a more generalized method called "Universal SpikeDeeptector" (USD), which is an extended version of SD. USD intends to detect and track the channels containing neural data recorded from four different species (rats, ravens, NHPs and humans) using different kinds of microelectrodes and different recording sites. To our knowledge, there is no method that can simultaneously detect and track neural data of multiple species. To enable contextual learning, USD constructs a feature vector from a batch of waveforms. The constructed feature vectors are then fed into a deep-learning algorithm, which learns contextualized, temporal and spatial patterns. USD is a supervised learning method. Therefore, it requires labeled data for training. It is mainly trained on data from a single human tetraplegic patient, and a small but equal portion of data from the remaining three species. The trained model is then evaluated on a test dataset collected from several humans, NHPs, rats, and birds. The results show that the USD performed consistently well across data collected from each species.

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Poster

090. Physiological Methods

Location: Hall A

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Program #/Poster #: 090.01/BB19

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Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from MEXT and AMED

KAKENHI 15H05723
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Senshin Medical Research Foundation

Title: Minimum aberration deep brain fluorescence microendoscopy using a miniature aspherical lens assembly

Authors: *M. SATO[1,2], S. SANO[3], H. WATANABE[3], J. NAKAI[1,4];
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Abstract: Fluorescence microendoscopy typically uses gradient refractive index (GRIN) lenses as optical probes. However, GRIN lenses inherently suffer from strong aberration and distortion effects that degrade the image quality and limit the effective field of view, respectively. Aspherical lenses, which change their radius of curvature with distance from the optical axis, can effectively eliminate spherical aberrations. The application of such lenses has not been explored in the field of fluorescence microendoscopy due to the technical difficulties presented in manufacturing miniature aspherical lenses. In this study, we have designed and characterized a novel miniature aspherical lens assembly for deep brain fluorescence microendoscopy. Our lens assembly, named "PAS probe," comprises of two nested pairs of aspherical lenses made through a precision glass molding process. The total length and diameter of the assembly are 7.06 and 0.6 mm, respectively. The assembly takes place in a stainless steel tube with an outer diameter of 0.7 mm. The assembly has a numerical aperture of 0.21, magnification factor of 1, and working distance of 0.15 mm. When fluorescent microspheres were imaged, the aspherical lens assembly exhibited markedly reduced image distortion and aberration when compared to the widely used GRIN lens. We then tested the usability of the PAS probe in the imaging of neuronal activity in the deep brain. C57BL/6 mice were subjected to an intrahippocampal CA1 microinjection of the AAV vector which drove the expression of the red fluorescent calcium indicator protein R-CaMP1.07 in neurons. After 4 weeks of recovery time, a stainless steel guide tube was then implanted. Two-photon imaging through the PAS probe at 1040 nm visualized spontaneous activity of the R-CaMP1.07-expressing neurons at a scan rate of 15 frames per second under isoflurane anesthesia. The results clearly demonstrated the capability of the PAS probe for fast-scanning two-photon microendoscopic imaging of subcortical neuronal activity, as well as the successful transmission of long wavelength near-infrared excitation light. Our study shows the practical advantage brought forth by aspherical microlenses in circumventing image distortions and aberration inherent to GRIN lenses, thus offering a new alternative for designing and manufacturing microendoscopic lenses. Developing a wide repertoire of aspherical microlens assemblies with varying diameters, lengths, and lens compositions will thus expand the potential of this technology.

Disclosures: **M. Sato:** None. **S. Sano:** A. Employment/Salary (full or part-time):; Sumita Optical Glass, Inc. **H. Watanabe:** A. Employment/Salary (full or part-time):; Sumita Optical Glass, Inc.. **J. Nakai:** None.

Poster

090. Physiological Methods

Location: Hall A

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Program #/Poster #: 090.02/BB20

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative U01 NS094247
NIH BRAIN Initiative R01 NS104944

Title: Improving Epac-based FRET sensors for imaging cAMP *in vivo*

Authors: *C. I. MASSENGILL, T. MAO, H. ZHONG;
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Abstract: Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger regulating a plethora of cellular events and biological processes, including synaptic plasticity, metabolism, and gene expression. This diversity is owed to the convergence of a variety of neuromodulators at G-protein-coupled receptors, whose signals are decoded through distinct spatiotemporal compartmentalization of second messengers within the cell. Yet, how cAMP integrates these heterogeneous neuromodulatory inputs in the intact organism is poorly understood, largely due to the lack of appropriate tools to visualize it *in vivo*.
Epac (exchange protein directly activated by cAMP) is one of the major downstream effectors in intracellular cAMP-mediated signaling pathways. Since Epacs exhibit large conformational changes in response to cAMP binding, they have been exploited to develop genetically-encoded cAMP sensors. By linking donor and acceptor fluorescent proteins to Epac's N- and C-termini, respectively, Förster resonance energy transfer (FRET) can be measured as a readout of cAMP concentration.
To achieve imaging *in vivo*, we assessed existing Epac-based sensors for their response to the neuromodulator norepinephrine using two-photon fluorescence lifetime imaging microscopy (2pFLIM), which offers advantages over other conventional FRET-quantifying measures, especially in light-scattering brain tissue. Furthermore, through structural analysis and site-directed mutagenesis, we generated new variants with significant improvements to sensitivity, dynamic range, subcellular localization, and aggregation. In ongoing work, we are developing viral reagents to express this series of sensors for application *in vivo*. We hope that our efforts will result in a suite of greatly improved sensors that allow for previously unattainable single-cell imaging of cAMP dynamics in awake mice.

Disclosures: **C.I. Massengill:** None. **T. Mao:** None. **H. Zhong:** None.

Poster

090. Physiological Methods

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KAKENHI 19H01142 to Takashi Tominaga
MHLW H30-Kagaku-Ippan-003 to Takashi Tominaga

Title: A fast intrinsic optical signal (FIOS) from unstained hippocampal slice is a novel kind of optical signal: comparison with the voltage-sensitive dye signal

Authors: Y. TOMINAGA[1], M. KOIKE-TANI[2], T. TANI[2], *T. TOMINAGA[1];
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[2]Eugene Bell Ctr. for Regenerative Biol. and Tissue Engin., Marine Biol. Lab., Woods Hole, MA

Abstract: A multipoint real-time recording from the brain tissue is necessary for decoding brain activity. Optical recordings are one of such technologies. Many optical imaging methods are available that use different signal sources. A type of intrinsic optical signal (IOS) from brain tissue is common in probing brain activity. Usually, IOSs have longer time constants and are practical for mapping activity upon specific brain functions because the origin of the signal mostly depends on metabolic and structural cascades. In contrast, optical recordings with voltage-sensitive dye (VSD) have been used to visualize faster signals depending on the membrane's potential changes in the neurons. VSD imaging (VSDI) has developed since its first description in the 1970s. The VSDI had been a very technically challenging method. We have been working on those developments, and the advances in the imaging, optics, and other supporting technique have made it a reliable measurement method comparable to the electrophysiological tools in a slice preparation of brain tissue. Here, we report that our improved methods are also beneficial in recording the intrinsic signal from unstained mammalian brain tissue. We have found that single electrical stimulation to Schaffer collateral elicited a fast but small (10-4) change in transmitted light along the stratum radiatum of area CA1 of the hippocampal slice. Since the intrinsic optical signal at that time course is new in brain slice preparation, we named it as the fast intrinsic optical signal (FIOS) and studied characteristics further. By comparison with the VSDI signal, we found that the FIOS start almost the same time

with the VSDI signal but took a longer time to reach to its peak (30 msec). The application of 50 µM APV did not affect FIOS while 10 µM CNQX diminished the FIOS. Application of 10 µM Gabazine enhanced the FIOS. Those pharmacological results were the same as those of the VSDI signal. Also, physiological modulation of synaptic connection such as paired-pulse facilitation and the long-term potentiation was seen in FIOS. Those results indicate that FIOS depends on the membrane potential response in post-synaptic cells and the possible use of FIOS as a measure of the brain activity with a non-invasive optical measurement.

Disclosures: **Y. Tominaga:** None. **M. Koike-Tani:** None. **T. Tani:** None. **T. Tominaga:** None.

Poster

090. Physiological Methods

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Title: Two-photon photoactivated voltage imaging in tissue with an archaerhodopsin-derived reporter

Authors: *D. BRINKS[1,2], M.-P. CHIEN[1,3], G. SILVA[1], Y. ADAM[1], W. BLOXHAM[1], S. KHEIFETS[1], A. E. COHEN[1];
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Abstract: Robust voltage imaging in tissue remains a technical challenge. Existing combinations of genetically encoded voltage indicators (GEVIs) and microscopy techniques cannot simultaneously achieve sufficiently high voltage sensitivity, background rejection, and time resolution for high-resolution mapping of sub-cellular voltage dynamics in intact brain tissue. We developed a pooled high-throughput screening approach to identify Archaerhodopsin mutants with unusual photophysical properties. After screening ~10^5 cells, we identified a novel GEVI, NovArch, whose 1-photon near infrared fluorescence is reversibly enhanced by weak 2-photon excitation. Because the 2-photon excitation acts catalytically rather than stoichiometrically, high fluorescence signals, optical sectioning, and high time resolution are achieved simultaneously, at modest 2-photon laser power. We developed a microscopy system

optimized for NovArch imaging in tissue. The combination of protein and optical engineering enhanced signal contrast sufficiently to enable optical mapping of back-propagating action potentials in dendrites in acute mouse brain slice.

Disclosures: D. Brinks: None. **M. Chien:** None. **G. Silva:** None. **Y. Adam:** None. **W. Bloxham:** None. **S. Kheifets:** None. **A.E. Cohen:** None.

Poster

090. Physiological Methods

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Shanghai Municipal Science and Technology Major Project (2018SHZDZX05)
National Key R&D Program of China (2017YFA0700500)

Title: Boosting sensitivity in light field microscopy for fast volumetric calcium imaging in living zebrafish brain

Authors: Z. ZHANG[1,2], L. CONG[1], F. LI[1,3], J. DU[1,3], *K. WANG[1,2];
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Abstract: Light field microscopy is a volumetric imaging technique that allows capture of 3D information simultaneously in just one exposure. Its scanning-free and highly parallelized imaging collection manners attract considerable interest in neuroscience studies where high throughput recording of functional activities over populations of neurons is essential. For example, we developed a new type of light field microscope with extended field of view (XLFM) and combined it with a fast 3D tracking system to record whole brain neural activities in freely swimming larval zebrafish during their natural prey capture behaviors (Cong et al. eLife 2017). However, the increased imaging speed of light field microscope comparing with other types of 3D imaging methods comes at costs of compromised spatial resolution and signal detection sensitivity.
Here, we present an optimally designed XLFM for whole zebrafish brain functional imaging. By carefully compensating off-axis optical aberrations induced by non-ideal imaging objective and relay lenses, we achieved 2 μm x 2 μm x 3 μm spatial resolution uniformly over a cylindrical imaging volume of Φ 800 μm x 200 μm and at a volume imaging rate of 6 Hz in newly designed

XLFM. Moreover, the new system is ~10 times more sensitive than our previous version in detecting fluorescence signal change of single neurons labelled with calcium sensitive fluorescent indicator GCamp6s. We also successfully combined this imaging system with fast 3D tracking system and achieved high resolution calcium imaging in freely behaving larval zebrafish. Enabled by the increased spatial resolution and sensitivity, we were able to identify much more subtle neural activity during natural behaving processes of zebrafish.

Disclosures: **Z. Zhang:** None. **L. Cong:** None. **F. Li:** None. **J. Du:** None. **K. Wang:** None.

Poster

090. Physiological Methods

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Topic: I.04. Physiological Methods

Support: NIH Grant R01 NS099429

Title: Speed considerations for large field two-photon microscopy

Authors: ***H. B. BANKS**, J. R. BUMSTEAD, L. M. BRIER, A. BICE, J. P. CULVER; Washington Univ. in St. Louis, St. Louis, MO

Abstract: With good optical design, a two-photon microscope (TPM) can image a large field of view (FOV) with high resolution [1], but running such a TPM at a high frame rate with an acceptable signal-to-noise ratio (SNR) requires careful consideration of the fluorophore and the mouse brain tissue. We present our strategy for achieving high frame rate and SNR in a large FOV TPM, the consequences of increasing the excitation laser power, and how the system expands the spatial and temporal bandwidth available to study functional connectivity. Our TPM is designed to be sensitive to the natural length scales of the mouse brain, having the resolution to measure single cells, about 10 microns, across nearly the entire cortex, about 5 mm. The speed considerations of the TPM are more complex. Genetically-encoded calcium-sensitive fluorophores such as GCaMP have inherent calcium binding/unbinding times, and fluorescence contrast ratios for calcium binding [2]. These properties set the minimum temporal resolution of the microscope as well as enforce a lower bound on the SNR in order to identify calcium transients robustly. The large space bandwidth product and the fast frame rate, however, require the pixel dwell time to be relatively small, limiting the integrated light from each pixel. To offset this drop in signal, we take advantage of the nonlinearities involved in the two-photon excitation process, in that increasing the laser power quadratically increases the fluorescence. Of course, increasing the power will, in turn, adversely affect the signal over time and the mouse itself. The fluorophores will bleach more quickly, and high laser power will increase the likelihood of photodamage and thermal damage to the brain. We show that these damage mechanisms,

however, all scale beneficially with increasing FOV, so that our nearly cortex-wide FOV moderates the effects of increasing the power, and the laser power can be increased safely and substantially. Finally, we show how these improvements in FOV, resolution, frame rate, and SNR can be used to develop a local understanding of large scale functional connectivity.
1. Jonathan R. Bumstead, et al., "Designing a large field-of-view two-photon microscope using optical invariant analysis" *Neurophotonics*, 5(2), 025001 (2018)
2. Hod Dana, et al., "Thy1-GCaMP6 Transgenic Mice for Neuronal Population Imaging *In Vivo*" *PLoS ONE*, 9(9) e108697 (2014)

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Poster

090. Physiological Methods

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Topic: I.04. Physiological Methods

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NSF Neuronex NEMONIC

Title: A next generation wide field-of-view multi-area two photon microscope for simultaneous imaging across mouse sensorimotor cortex

Authors: *M. A. CLOUGH[1], I. A. CHEN[1], S. L. SMITH[3], J. L. CHEN[2];
[1]Biomed. Engin., [2]Boston Univ., Boston, MA; [3]Electrical and Computer Engin., UC Santa Barbara, Santa Barbara, CA

Abstract: Simultaneous recording of neural activity across cortical areas during a behavioral task can provide insight into how information is processed and communicated between cortical areas. Sensory and motor cortices can be separated by >4.5mm in the mouse brain. The large separation between these areas makes it difficult to simultaneously record population activity with cellular resolution at high temporal resolutions from each area using standard imaging systems. To address this gap we have designed a next generation multi-area two-photon microscope with a field of view large enough to image across mouse sensorimotor cortex. This system was based on a combination of two previously developed systems, a first generation multi-area two-photon microscope (Chen, Voigt et al. 2016) and the Trepan2P microscope (Stirman et al. 2016). A combination of commercial and custom-designed optics was used to achieve a 4.7mm field of view at ~0.52 NA. Excitation light is driven by a 31.25MHz 1040nm Ytterbium fiber laser optimized for two-photon imaging of red calcium indicators which enables

spatiotemporal multiplexing of four sub-areas at high frame rates (>30Hz). Independent steering of each of the four beams was achieved by the use of 'focal plane units' coupled to two independent scan engines to provide x-y positioning and electrically tunable lenses to provide z-positioning. This new imaging system will enable a better understanding of cortex-wide population dynamics that emerge during behavior.

Chen, Jerry L, et al. "Long-Range Population Dynamics of Anatomically Defined Neocortical Networks." *ELife*, 24 May 2016, doi:10.7554/elife.14679.
Stirman, Jeffrey N, et al. "Wide Field-of-View, Multi-Region, Two-Photon Imaging of Neuronal Activity in the Mammalian Brain." Nature Biotechnology, vol. 34, no. 8, 27 June 2016, pp. 857-862., doi:10.1038/nbt.3594.

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Poster

090. Physiological Methods

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 090.08/BB26

Topic: I.04. Physiological Methods

Support: NIH Grant R00NS079471
University of Pittsburgh

Title: Resting state connectivity revealed with intrinsic signal optical imaging in squirrel monkeys

Authors: *N. S. CARD[1], O. A. GHARBAWIE[2];
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Abstract: Determining the organization of brain networks is a foundational problem in systems neuroscience. Correlating spontaneous fluctuations in hemodynamic signals as recorded with fMRI, i.e. *resting state connectivity*, has become one of the leading approaches for defining brain connectivity in primates. The approach has been particularly instructive in revealing the organization of cortical networks at the mm-to-cm scale. However, whether spontaneous fluctuations in hemodynamic signals could be exploited for resolving subtler network components (μm-to-mm scale) is less clear. Revealing connectivity at this scale would enable the mapping of cortical networks at the level of cortical columns, thus approaching the resolution of neuroanatomical tracers. Towards that objective, we used intrinsic signal optical imaging (ISOI, 630 nm illumination) to measure spontaneous fluctuations in frontal and parietal cortex of 3 squirrel monkeys. High resolution optical images (1312 x 1082 pixels, ~13 μm/pixel, 12-bits) were acquired in multiple sessions (15 min/session, 10 frames/s) from each animal. Optical images were temporally binned into 2 Hz time series and filtered (bandpass 0.01-0.1 Hz) for

cross-correlations between pixels. The optical window (~ 20 x 15 mm) included motor and somatosensory areas that were mapped with intracortical microstimulation and multi-unit recordings, respectively. Seeds for measuring *resting state connectivity* were strategically placed in representations of the arm and hand in frontal motor areas (M1, PMd, PMv) and in somatosensory areas 3b, 1, and 2. In separate imaging sessions, *effective connectivity* was determined for the same seeds using microstimulation + ISOI, which revealed postsynaptic targets at submillimeter scale. Co-registration of the *resting state* maps with the somatotopic maps showed correspondence between *resting state connectivity* and cortical networks traced anatomically in previous work. Moreover, spatial correspondence was apparent between *resting state connectivity* and *effective connectivity*. Two notable differences between the 2 approaches: (1) every spatial patch within the *resting state* map was larger than the corresponding patch in the *effective connectivity* map; (2) *resting state* maps varied more between sessions and monkeys as compared to *effective connectivity* maps. Nevertheless, the results show that ISOI can reveal *resting state connectivity* at high spatial resolution. The results have prompted us to record electrophysiological signals from connected patches in an effort to interrogate the neural activity that could underlie *resting state connectivity*.

Disclosures: N.S. Card: None. **O.A. Gharbawie:** None.

Poster

090. Physiological Methods

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Topic: I.04. Physiological Methods

Support: NIH grant R21EY026425
NIH grant F32MH115432

Title: A dual-mode FRET and BRET sensor for monitoring cAMP dynamics

Authors: *A. R. FRENCH, A. L. TESMER, M. TANTAMA;
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Abstract: Genetically encoded indicators (GEIs) are engineered proteins that convert physiological signals, such as the concentration of a molecule or membrane voltage, to a change in fluorescence or bioluminescence. These tools have revolutionized our ability to monitor signaling events in tissues such as the CNS. One interesting application of GEIs is in dissecting signaling events downstream of GPCRs, which are key targets for pharmaceuticals. Ideally, a single GEI could be used for screening GPCR drug candidates and for validating the drug's mechanism *in vivo* and *in vitro*. However, current GEIs are limited by their respective modes of action. Though GEIs utilizing fluorescence resonance energy transfer (FRET) are excellent at

dissecting subcellular GPCR signaling dynamics *in vitro*, their use *in vivo* is limited by high scattering of the fluorescence excitation energy in tissue. Similarly, GEIs using bioluminescence resonance energy transfer (BRET) have been successfully used for cell-based drug screening *in vitro*, but their application *in vivo* is limited by the brightness of the luciferases in current GEIs. NanoLuc, an exceptionally bright shrimp luciferase, could potentially alleviate this problem for BRET-based GEIs. We therefore sought an approach to engineering GEIs that would combine the benefits of fluorescence and bright NanoLuc bioluminescence. We hypothesized that enhanced Nano Lanterns (eNLs), NanoLuc-fluorescent protein fusions capable of both FRET and BRET, could be rapidly integrated into GEIs to generate sensors that can operate both through bright NanoLuc-based BRET and high-resolution FRET. As a proof of concept, we tested our approach on a FRET-based GEI that senses cyclic adenosine monophosphate (cAMP), a secondary messenger in G-protein-dependent GPCR signaling. We demonstrate that our sensor responds to drug-induced changes in cellular cAMP in both FRET and BRET modes with a dynamic range comparable to that of previous cAMP sensors. We also show that our sensor is bright enough to be used in mouse tissue as a ratiometric BRET sensor.

Disclosures: **A.R. French:** None. **A.L. Tesmer:** None. **M. Tantama:** None.

Poster

090. Physiological Methods

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Program #/Poster #: 090.10/BB28

Topic: I.04. Physiological Methods

Support: Bill and Melinda Gates Foundation OPP1184813
NIH NINDS R01NS090874
McDonnell Cognitive Computational and Systems Neuroscience Fellowship
NIH NICHD U54HD087011

Title: A new high density diffuse optical tomography system for functional brain mapping in preschool-age children

Authors: *K. TRIPATHY[1], A. M. SVOBODA[2], M. L. SCHROEDER[2], A. K. FISHELL[3], E. J. RICHTER[6], S. RAFFERTY[4], C. TRACY[4], Z. E. MARKOW[2], M. WHEELOCK[4], A. T. EGGEBRECHT[5], J. P. CULVER[7];
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Abstract: Developmental neuroimaging studies have been constrained by limitations of extant imaging modalities. While functional magnetic resonance imaging (fMRI) has revolutionized cognitive neuroscience, it is challenging to train young children to remain calm, still, and cooperative during tasks when alone inside an MRI scanner, and children younger than 4 years can usually only be imaged while asleep. Other methods like functional near infrared spectroscopy (fNIRS) may be used to image children in a more open, child-friendly scanning environment, but produce data of lower spatial resolution and image quality. High density diffuse optical tomography (HD-DOT) is a high performance optical neuroimaging method that uses a dense array of overlapping light measurements to enhance image quality over traditional fNIRS and has been validated extensively against fMRI in adults. However, HD-DOT is yet to be evaluated for imaging in awake young children. Here we present a new HD-DOT system that we built for imaging preschoolers, with a 128-source by 125-detector console, lighter fiber optics, structural improvements to increase wearability, an expanded field-of-view, and a child-friendly imaging suite. We validate our new system by mapping expected cortical activations during visual, auditory, and motor tasks, and further quantitatively benchmark the system against one of our previously established HD-DOT systems using visual task data from participants imaged on both systems. We then use the new system to image both adults and 2-5 year-old children while they watch animated movie clips, since head motion, which is notorious for creating artifactual findings in neuroimaging studies, is reduced in young children during movie viewing. We compare data across repeated presentations of a movie, replicating with our new imaging system the finding from prior fMRI research that movie viewing evokes reproducible patterns of brain activity in both adults and children. Finally, we compute voxelwise correlations with movie features such as audio intensity and speech content, and show that these regressors can be used to map associated brain areas from our movie viewing data. These results show the promise of HD-DOT for mapping brain development in preschool age children.

Disclosures: **K. Tripathy:** None. **A.M. Svoboda:** None. **M.L. Schroeder:** None. **A.K. Fishell:** None. **S. Rafferty:** None. **C. Tracy:** None. **M. Wheelock:** None. **Z.E. Markow:** None. **E.J. Richter:** None. **A.T. Eggebrecht:** None. **J.P. Culver:** None.

Poster

090. Physiological Methods

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 090.11/BB29

Topic: I.04. Physiological Methods

Title: High-throughput microscopy platform toward a roadmap for accelerating vertebrate neuroregeneration research

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Abstract: Injuries to central nervous system (CNS) are prevalent and lack effective therapies. A conditioning lesion of the peripheral sensory axon triggers robust central axon regeneration in mammals, suggesting that lesion conditioning could drive powerful therapies for CNS injuries. We established a model for lesion-conditioned regeneration in the ASJ neuron of the invertebrate roundworm *C. elegans*. This approach enables a faster search for neuronal regeneration genes compared to mammalian models. Even so, a complete search for regeneration genes requires surgery and reimaging of a fluorescent neuron in >30,000 animals, which is unfeasible. A high-throughput surgery and reimaging platform for rapid immobilization, imaging, and optical surgery of *C. elegans,* could enable this gene search in a reasonable timeframe. A complete regeneration gene search requires the acceleration of two time-consuming steps: animal immobilization and precise neuronal microscopy. This can be accomplished through two specific goals. The first goal is to develop a microscope stage to readily immobilize many animals for *in vivo* imaging. *C. elegans* will be immobilized by cooling them directly on their cultivation plates, instead of mounting them to slides. We finished the thermal simulation of the cooling stage and tested the cooling performance under experimental conditions, which confirmed the feasibility of immobilizing worms on their cultivation plates. The second goal is to enable robust automation of microscopy by improving neuron-background contrast. Patterned illumination approaches will enhance contrast by reducing the bright haze around the cell body and exposing the axon and dendrites nearby. We developed a new image acquisition algorithm and utilized a spatial light modulator for illumination patterning. The accomplishment of our goals will pave the path to a high-throughput microscope platform for *C. elegans* research, which can be used in numerous neuroscience labs worldwide.

Disclosures: Y.L. Wang: None. **E.L. Jaklitsch:** None. **S.H. Chung:** None.

Poster

090. Physiological Methods

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Program #/Poster #: 090.12/BB30

Topic: I.04. Physiological Methods

Support: NIH
HHMI
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Title: Multi-axis two-photon microscopy for imaging neural activity across distributed circuits in behaving animals

Authors: *T. H. KIM[1,2,6], M. J. WAGNER[3], O. JAIDAR[4], Y. ZHANG[1,6], J. B. DING[4], L. LUO[3,6], M. J. SCHNITZER[1,3,5,6];
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Abstract: A major technological goal in neuroscience is to image the simultaneous dynamics of anatomically connected neural populations throughout the brain. Two-photon microscopy allows observations of the activity patterns of genetically identified or projection-targeted neurons in the live mammalian brain, but has generally been limited to individual optical fields-of-views. Here, we present a multi-axis approach to two-photon microscopy that provides concurrent optical access to multiple deep or superficial brain areas in awake behaving animals. With this approach we performed long-term imaging studies of the shared dynamics between motor cortical pyramidal neurons and cerebellar granule cells in mice learning to perform forelimb manipulation of a robotic manipulandum[1]. The combination of dual-axis two-photon microscopy and optogenetic manipulations revealed the necessity of pontine projections to the cerebellum for the shared corticocerebellar activity patterns. These results highlight the potency of the multi-axis imaging approach for studying the interactions between anatomically separated brain areas with coordinated dynamics.
Recently, we extended our published dual-axis two-photon microscope[2] to enable dual-color fluorescence calcium imaging with independent depth control at each of the two fields-of-views, thereby enabling functional readouts from two distinctly defined cell populations at each of the two imaging sites. We have also identified key optomechanical and surgical techniques to extend our multi-axis imaging approach to four or more brain areas in a single animal subject. To this end, we miniaturized our two-photon imaging instrumentation to allow a dense packing of several micro-optical objective lenses upon the cranium. For imaging deep brain areas, we combine the use of these tiny objective lenses with microendoscopes implanted into brain tissue. Finally, we used techniques from surgical robotics to create a set of miniaturized two-photon imaging heads that can be independently and accurately manipulated by remote-center-of-motion actuation. Together, these engineering approaches will enable optical studies of four or more brain areas simultaneously in behaving mice.
[1]Wagner*, Kim* *et al.* Cell (2019). [2]Lecoq *et al.* Nat. Neurosci (2014).

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Poster

090. Physiological Methods

Location: Hall A

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Program #/Poster #: 090.13/BB31

Topic: I.04. Physiological Methods

Title: A modular open-source framework for two-photon and lightsheet microscopes

Authors: *M. NIKITCHENKO[1], E. A. NAUMANN[3], E. E. THOMSON[2], M. LORING[1]; [1]Neurobio., [2]Duke Univ., Durham, NC; [3]Neurobio., Duke Univ. Sch. of Med., Durham, NC

Abstract: Here we present a user-friendly, modular, open-source framework for building two-photon and lightsheet microscopes. To develop easily replicable microscopes for flexible use in structural and functional imaging as well as holographic optogenetic photostimulation, we provide well-documented 3D models, and a complete parts lists with explanations of multiple options for customization. We also provide open-source Python and LabView code libraries to operate all associated hardware in a modular framework. This modularity facilitates the construction of updates and expansions, while multiple implementations of the modules provide a degree of protection against hardware components going obsolete. We also designed a workflow that allows for the semi-automated ordering of hardware, as well as the assembly and installation of software in a short period of time. The designs can be implemented by either using exclusively off-the-shelf components, or by building DIY replacements which significantly reduce the overall cost. In some cases, the custom-built components provide a substantial improvement over the commercially available components. We hope that this open source, modular design will invite an incorporation of new methodologies into the framework. While the current designs are tailored to functional imaging in zebrafish, they require only minimal change to be used with other animal models.

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Poster

090. Physiological Methods

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Cognitive, Computational, and Systems Neuroscience Fellowship, McDonnell Center, Washington Univ. In St. Louis

Title: Developing diffuse optical tomography with multiple cameras for imaging mouse brain hemodynamics in deep and superficial tissue

Authors: *Z. E. MARKOW[1], M. D. REISMAN[2], A. Q. BAUER[2], A. T. EGGEBRECHT[2], M. A. ANASTASIO[3], J. P. CULVER[2];
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Abstract: Current optical systems for functional neuroimaging in mice over the whole cortical area are limited to tissue near the brain surface and require surgery to remove the scalp and thin or remove the skull. Diffuse optical tomography (DOT) can overcome these challenges by inverting a physical model of tissue illumination to reconstruct three-dimensional (3D) images of brain activity-related changes in blood oxygenation. However, previous rodent DOT systems provided insufficient imaging speeds, had low resolution, or had limited 3D fields of view for functional neuroimaging. To solve these problems, we have constructed a new mouse brain DOT system using <100 structured light patterns for broad illumination and sufficiently fast imaging (1-10 Hz) and multiple sCMOS cameras with >1000 detectors for dense spatial sampling and an extensive 3D field of view. Illumination is performed with digital micromirror devices, projectors, and optics that support up to 6 wavelengths. To coregister the mouse head and camera measurements, we developed a surface profiling method in which points of light are scanned over a flat object in a one-time calibration session and then are scanned over the mouse head at the beginning of each mouse functional neuroimaging session. Using simple linear algebra, we infer the light source beam directions from the calibration data and combine this with the mouse scan data to coregister the images from all cameras and make 3D mesh models of the head. For validation, we 3D-printed a statuette from a known mesh and performed our surface profiling method on the statuette. The surface vertices of our data-derived mesh had position errors 0.4 ± 0.2 mm (mean ± SD) from the known mesh. We then reconstructed simulated activations at multiple depths within a mouse head mesh from our surface profiling method to determine the imaging system's field of view, sensitivity, and resolution versus depth. This showed sensitivity to blood oxygenation changes at depths >4 mm and resolution (spatial blurring) ≤1.0 times the depth. For in-vivo validation, we imaged neural responses to forepaw stimuli using 1 projector, 2 wavelengths, and 1 camera of this imaging system in 5 mice. Results also suggest that fewer wavelengths could be employed to increase imaging speed.

Disclosures: Z.E. Markow: None. **M.D. Reisman:** None. **A.Q. Bauer:** None. **A.T. Eggebrecht:** None. **M.A. Anastasio:** None. **J.P. Culver:** None.

Poster

090. Physiological Methods

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Program #/Poster #: 090.15/BB33

Topic: I.04. Physiological Methods

Support: R01 MH111520
NS081707
K01 DA042219

Title: Photoswitchable GPCR-based opsins for *in vivo* subcellular neuromodulation

Authors: *B. A. COPITS[1], P. O'NEILL[2], J. YOO[1], A. VASQUEZ[3], V. K. SAMINENI[5], C. STANDER[1], R. K. SUNAHARA[4], R. W. GEREAU, IV[6], M. R. BRUCHAS[7];
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Abstract: The development of diverse molecular tools to manipulate the activity of defined cell types has greatly accelerated our understanding of the neural circuits underlying complex behaviors. While optogenetic approaches can control entire neuronal populations at millisecond timescales, this may not accurately reflect circuit dynamics. In contrast, the use of chemogenetic tools to engage endogenous signaling cascades may produce more physiological changes in activity, however they are spatially and temporally constrained by their exogenous ligands. The development of rhodopsin-GPCR (opto-XR) chimeras grants increased spatial and temporal control of distinct intracellular cascades, and in contrast to binary on/off manipulations, mimicking these signaling pathways may more precisely mirror circuit function *in vivo*. However, these rhodopsin-based approaches possess several limitations, including high photosensitivity and irreversible activation.
Here we have identified a novel photoswitchable GPCR-based opsin that engagesendogenous inhibitory signaling cascades to silence synaptic transmission. ThisUV/blue light-sensitive opsin permits long-term optical inhibition with pulsed light, does not desensitize, and is rapidly reversed by illumination with amber/red light. This opsin can also be stimulated by multiphoton excitation, permitting subcellular activation of G-protein subunits. These unique features may allow for spatiotemporal control of GPCR signaling in subsets of cells within a circuit or distinct neuronal compartments, like spines vs dendrites, *in vivo*. We are also adapting this GPCR-based opsin to serve as a novel scaffold for next-generation opto-XR chimeras for photoswitchable control of neuromodulatory signaling, to better understand how these important therapeutic targets modulate circuit dynamics and brain function.

Disclosures: B.A. Copits: None. **P. O'Neill:** None. **J. Yoo:** None. **A. Vasquez:** None. **V.K. Samineni:** None. **C. Stander:** None. **R.K. Sunahara:** None. **R.W. Gereau:** None. **M.R. Bruchas:** None.

Poster

090. Physiological Methods

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Support: NIH 5R01HL126747-03
NIH 3OT2OD025307-01S1

Title: Combining infrared neuromodulation (IRN) with isotonic glucose solution to lower the IR dose requirement

Authors: *J. ZHUO[1], M. T. MCPHEETERS[1], E. M. JACKSON[2], S. S. SHANKAR[1], M. GANGULY[5], E. D. JANSEN[5,6], H. J. CHIEL[2,1,3], M. W. JENKINS[1,4];
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Abstract: Better treatments are needed for diseases related to the autonomic nervous system or the immune system (e.g., rheumatoid arthritis, obesity, heart failure). Modulating sensory information carried by small-diameter fibers in these systems could be an effective treatment strategy. Previously, we have shown that infrared neuromodulation (IRN) can preferentially modulate peripheral sensory fibers. To increase the utility and safety of IRN, we are actively studying methods to lower the dose requirement. Isotonic glucose contains none of the ions necessary to sustain an action potential. At sufficiently high concentrations, isotonic glucose entirely blocks action potential propagation. Therefore, we hypothesize that combining glucose with low levels of IRN will inhibit small-diameter axons.
To test our hypothesis, we stimulated and recorded compound action potentials (CAPs) from the pleural-abdominal connective of *Aplysia californica*, using suction electrodes on each end. This nerve's length allows the different components (by conduction velocity) of the CAP to separate from each other during propagation. We electrically stimulated the nerve at 1 Hz with 350 us pulse width and 0.7 - 1.5 mA stimulation current range (current level was determined individually to ensure full recruitment of the CAPs). The nerve was placed across a triple-chamber platform. Each chamber was sealed from the others, but the nerve could course through all three. *Aplysia* saline was placed in the two outer chambers while the middle testing chamber was perfused with *Aplysia* saline, isotonic glucose solution (20.41 w/v %) or a mixture of both. The glucose concentration in the middle chamber is varied during the test and monitored with an inline conductivity meter. We tested the glucose concentration threshold for complete inhibition of all electrically stimulated CAPs. We also tested the IRN radiant exposure threshold for complete inhibition in normal *Aplysia* saline. Control experiments were conducted before and

after each test to assess the nerve's health. With both thresholds established, we examined the combination of sub-threshold IRN with sub-threshold glucose solution to see if we could achieve complete inhibition of the CAPs. The results confirmed our hypothesis that combining IRN with glucose solution can lower the required radiant energy for inhibition which can increase translational potential.

Disclosures: J. Zhuo: None. **M.T. McPheeters:** None. **E.M. Jackson:** None. **S.S. Shankar:** None. **M. Ganguly:** None. **E.D. Jansen:** None. **H.J. Chiel:** None. **M.W. Jenkins:** None.

Poster

090. Physiological Methods

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Topic: I.04. Physiological Methods

Support: BRAIN U19
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Title: Cell-type specific optical recordings of electrophysiological oscillations in behaving mice using genetically encoded fluorescent voltage indicators

Authors: *S. HAZIZA[1], R. CHRAPKIEWICZ[1], J. MARSHALL[2], M. KANNAN[3], G. VASAN[3], K. CHO[4], T. DAVIDSON[4], T. TASCI[1], J. LI[1], Y. ZHANG[1], J.-Z. LI[1], V. PIERIBONE[3], M. LIN[1], V. SOHAL[4], M. SCHNITZER[1];
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Abstract: Electrophysiological field potential oscillations hold widespread interest in basic and clinical neuroscience, as specific types of oscillations are often indicative of particular brain and behavioral states in both health and disease. However, for many forms of voltage oscillation, the underlying cellular and network mechanisms remain poorly understood. The experimental challenges to dissecting these mechanisms include the inability of field electrodes to differentiate the contributions of specific cell-types, as well as interpretative confounds, such as volume conduction effects arising distal to the location of field electrode recordings. To address these challenges, here we present an optical technique that uses genetically encoded fluorescent voltage indicators to perform recordings of aggregate, neural population dynamics in awake behaving animals. These recordings achieve cell-type specificity and report neural trans-membrane potential dynamics, rather than the extracellular potentials measured in electric field recordings. Our technique is termed TEMPO (Trans-membrane electrical measurements performed optically) and attains a measurement sensitivity at the physical limits set by photon shot noise. To achieve this exquisite sensitivity, TEMPO combines: (i) a reference fluorescence

channel to track hemodynamic and brain motion artifacts; (ii) phase-sensitive optical detection of fluorescence signals from the voltage indicator and the reference fluorophore; (iii) computational un-mixing of laser intensity fluctuations and biological artifacts from the voltage activity traces. We routinely apply TEMPO to study a range of cortical and sub-cortical brain areas in behaving mice using either green or red fluorescent voltage indicators. For example, by using two TEMPO apparatus we recently examined the extent to which gamma-band activity is synchronized across the cerebral hemispheres of behaving mice in parvalbumin-positive neocortical interneurons. We are now further developing the TEMPO approach to track multiple brain areas, the spatial dynamics of population voltage rhythms, and more than one neuron-type at a time. Overall, TEMPO provides a widely applicable experimental approach allowing the deconstruction of normal and pathologic neurophysiological states into the trans-membrane voltage activity patterns of specific cell types.

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Poster

090. Physiological Methods

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Topic: I.04. Physiological Methods

Support: Canadian Institutes for Health Research CIHR Foundation
Canadian Partnership for Stroke Recovery
Canadian Neurophotonics Platform
NIH R21

Title: Automated task training and longitudinal monitoring of mouse mesoscale cortical circuits using homecages

Authors: *T. H. MURPHY[1], J. LEDUE[1], M. BALBI[3], F. BOLANOS[4], M. P. VANNI[5], D. BIERBRAUER[1], T. SIU[1], L. A. BOLANOS[2], J. D. BOYD[1];
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Abstract: We report improved automated methodology for training mice and acquiring cortical mesoscale functional connectivity from mice. An updated head-fixation mechanism using Raspberry Pi-based hardware employs a servomotor which leads to significantly longer durations

of daily head fixation than previous solenoid-based systems (>4X) that we previously reported (Murphy et al. 2016 Nature Commun). The use of linked cages enables simultaneous training of 8-10 mice, as well as automatic animal weighing using RFID. We report results on training 5 cages of mice (44 total male GCAMP6 mice). We observed average of 28+-17 head fixations per day for a subset of animals tested (23/44) that exhibited high rates of headfixation. The time headfixed for these 23 good performers averaged 16+-20 min/day. Addition of a capacitive touch sensor to the water delivery spout allowed development of tasks based on cued licking and lick withholding. We define a staged protocol using partial and probabilistic restraint which allows mice to adjust to self-initiated automated headfixation over 3 weeks' time. Using GCaMP6 transgenic mice we have examined the activation of mesoscale cortical circuits during a task where mice wait for a tactile-vibration cue and respond by licking after a delay (9 of 23 mice achieved >70% success rate). Using a RGB camera, green epifluorescence in response to 473 nm light was used to report GCaMP6 activity, while 440 nm reflectance into the blue channel was used to report blood and movement artefact contamination and correct the functional signals. While automatically head fixed, we acquire spontaneous movement-triggered GCaMP6 signals, or task-evoked signals and employ an analysis pipeline to mark both behavioral events, as well as analyze fluorescence signals as they relate to spontaneous and/or task-evoked brain activity. UDP triggering, with time and mouse tag information encoded, of ancillary video recording provided a number of possible viewpoints of mouse behaviour to be recorded concurrently with the brain imaging. We have trained animals on a go and no-go task within their homecage. Correct go-trials were associated with activation in limb/trunk sensory areas during the vibration cue and delayed frontal and lateral motor activation consistent with reports of licking-related circuitry. This system provides an open-source platform to train mice to self-initiate headfixation and supports subsequent cue-based task training under simultaneous mesoscale brain imaging and is ideally suited for longitudinal modeling of human disease in mice.

Disclosures: T.H. Murphy: None. **J. LeDue:** None. **M. Balbi:** None. **F. Bolanos:** None. **M.P. Vanni:** None. **D. Bierbrauer:** None. **T. Siu:** None. **L.A. Bolanos:** None. **J.D. Boyd:** None.

Poster

090. Physiological Methods

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 090.19/BB37

Topic: I.04. Physiological Methods

Title: Clear optically matched panoramic access channel technique (COMPACT) for large volume deep brain imaging

Authors: *M. CUI;
ECE and Biol., Purdue Univ., West Lafayette, IN

Abstract: With the advance of sensitive molecular indicators and actuators, neurophotonics has become a powerful paradigm for discovering the principle of neural circuit. However, a major limitation of using light to study mammalian brain is the limited access depth. Even with the advance of multiphoton microscopy, the majority of implementation in mammalian brain is limited to ~ 1 mm depth, which is about the thickness of mouse neocortex. The majority of mouse brain remains inaccessible, not to mention the brain of larger mammals. To investigate deeper brain regions, invasive miniature optical probes are required. A key issue with the available optical probes is the tiny access volume, which is only a small fraction of the inserted probe volume. In fact, the design of miniature optical probes has largely remained the same over the past two decades. Can we fundamentally change the design of miniature optical probes and greatly increase the access volume? Here we report our latest invention, Clear Optically Matched Panoramic Access Channel Technique (COMPACT), which can effectively increase the tissue access volume by ~ **three orders of magnitude**. The development of COMPACT will provide tremendous capacity, flexibility and adaptability to the neuroscience research. We will discuss our experiment on employing COMPACT for one-photon and two-photon calcium imaging of deep mammalian brain.

Disclosures: M. Cui: None.

Poster

090. Physiological Methods

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 090.20/BB38

Topic: I.04. Physiological Methods

Support: NSF NeuroNex 1707352

Title: Miniaturized devices for bioluminescence imaging of brain and spinal cord

Authors: *D. CELINSKIS[1,3], M. GOMEZ-RAMIREZ[2], D. A. BORTON[1,3,4], C. I. MOORE[2,3]; [1]Sch. of Engin., [2]Neurosci., Brown Univ., Providence, RI; [3]Carney Inst. for Brain Sci., Providence, RI; [4]Dept. of Veterans Affairs, Providence Med. Center, Ctr. for Neurorestoration and Neurotechnology, Providence, RI

Abstract: Over recent years miniature microscopes have become a key tool for *in vivo* free behavioral studies. This technology offers the ability to measure network dynamics underlying naturalistic animal behaviors by tracking fluorescent activity of genetically encoded calcium indicators. However, these indicators suffer from autofluorescence artifacts, phototoxicity and photobleaching effects, limiting chronic animal studies. These limitations can be overcome by the use of bioluminescent indicators, which offer a lower biological noise floor and do not employ external light delivery. While often significantly higher in signal-to-noise ratio, the

overall lower signal intensity of luciferase output is a challenge. As such, imaging calcium or other cofactor-dependent bioluminescent indicators requires minimization of emission light losses, and optimization of the imaging sensor's sensitivity.
Here, we demonstrate a modified version of the open-source UCLA miniscope for bioluminescence imaging. The removal of excitation light optics reduces the number of assembly components by ~60%, and weight by ~20%. The reduction in components and the use of high-resolution stereolitographic (SLA) 3D printing also provides significant flexibility for the optical path design optimization to reduce the photon loss, to add complementary functions such as electrical stimulation or recording, and to adapt the microscope for imaging other parts of the body such as the spinal cord.
The studies employing miniature microscopes to date have been primarily focused on brain imaging and existing designs have not been optimized for chronic use to image the spinal cord. Key challenges remain when imaging the spinal cord, in particular significant movement artifact. Here, we will also describe an implant design for attaching the miniature microscope to the spinal cord, and demonstrate the adaptation of motion correction algorithms for spinal cord imaging applications.
We are currently validating the above described innovations by demonstrating bioluminescence imaging in the spinal cord of the mouse. A major goal of our project is to expand the utility of open-source miniature microscopes to new avenues of scientific inquiry (e.g., spinal cord imaging). As part of the technology sharing objectives of this NSF Bioluminescence Hub, all designs presented here are available online for use by all members of the scientific community.

Disclosures: **D. Celinskis:** None. **M. Gomez-Ramirez:** None. **D.A. Borton:** None. **C.I. Moore:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.01/BB39

Topic: B.08. Intrinsic Membrane Properties

Support: JSPS KAKENHI JP19J01590
MEXT/JSPS KAKENHI JP16H04663
MEXT/JSPS KAKENHI JP17K19451
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Brain/MINDS from AMED JP18dm0207064
JST ERATO JPMJER1801
JSPS Grants-in-Aid for Scientific Research 18H05525

Title: Fluorescent glass pipettes for optically targeted electrophysiological recordings

Authors: *K. OKAMOTO[1,2], N. FUJI[6], T. EBINA[3], K. KONISHI[4], Y. SATO[2], T. KASHIMA[2], R. NAKANO[2], H. HIOKI[1], H. TAKEUCHI[2], J. YUMOTO[4,5], M. MATSUZAKI[3], Y. IKEGAYA[2];
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Abstract: Targeted patch-clamp recordings are one of the major techniques to record intracellular physiological activity from a specific neuron. The method is often combined with genetical fluorescent cell labeling; however, glass pipettes have no emission in the visible light spectra, which makes it difficult to achieve targeted patch-clamp recordings. Here, we invented new fluorescent glass containing terbium(III), a rare earth ion. Pipettes made of the glass emitted green fluorescence upon 488-nm-light excitation and were visible in the same field of view with green fluorescent proteins. In addition, the pipettes exhibited strong third harmonic generation upon 1300-nm-laser excitation. Thus, our terbium(III)-doped pipettes can improve the performance of targeted patch-clamp recordings under both in vitro and in vivo conditions.

Disclosures: K. Okamoto: None. **N. Fuji:** None. **T. Ebina:** None. **K. Konishi:** None. **Y. Sato:** None. **T. Kashima:** None. **R. Nakano:** None. **H. Hioki:** None. **H. Takeuchi:** None. **J. Yumoto:** None. **M. Matsuzaki:** None. **Y. Ikegaya:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.02/BB40

Topic: I.04. Physiological Methods

Support: NIH R01NS102727
NIH Brain Initiative U01-MH106027-01

Title: High throughput opsin screening using automated intracellular electrophysiology

Authors: *M. C. YIP[1], C. R. LANDRY[2], A. YANG[3], I. KOLB[5], E. S. BOYDEN[3,4], C. R. FOREST[1];
[1]Woodruff Sch. of Mechanical Engin., [2]Wallace H. Coulter Sch. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Media Lab., [4]McGovern Institute, Departments of Biol. Engin. and Brain and Cognitive Sci., MIT, Cambridge, MA; [5]Janelia Res. Campus, Ashburn, VA

Abstract: While the need for high-throughput screening platforms has led to the development of massively parallelized robotic tools to perform a variety of cellular assays, manual and laborious

patch clamp recording has remained a necessary but critically limiting step in screening for genetic constructs that control or report the electrical properties of cells. The PatcherBot, an artificial intelligence-guided robot capable of performing dozens of patch recordings in cultured cells and brain slices without human intervention, is capable of using a single pipette to patch 12-15 cells per hour in a closed-loop process that can run unattended for up to 3 hours. This technology represents more than 10x throughput of genetic screens that rely on manual patch clamping to report biophysical properties. To wit, the red-shifted opsin Chrimson was discovered from ~1000 total whole cell patches from a library of ~120 variants, where each variant had to be screened independently in separate dishes of cells (i.e., number of variants per experiment = 1), with multiple replicates of each construct (n = ~8 whole cell patches per variant). Here, we show that the PatcherBot can dramatically improve the throughput and performance of both traditional and pooled genetic screens and is broadly applicable to the directed evolution of genetically-encoded sensors and actuators. Specifically, we show that a single manipulator version of the PatcherBot can enable unattended screening (i.e., fluorescence imaging, whole cell recording, and light stimulation) of up to 80 cells a day. This increased throughput, towards high-fidelity recordings, significantly increases both the size of libraries that can be screened, and the number of high-performance variants identified in a single experiment.

Disclosures: **M.C. Yip:** None. **C.R. Landry:** None. **A. Yang:** None. **I. Kolb:** None. **E.S. Boyden:** None. **C.R. Forest:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.03/BB41

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative Grant 1-U01-MH106027
NIH R01NS102727

Title: Patch-walking: Coordinated multi-pipette patch clamping to efficiently discover synaptic connections

Authors: M. C. YIP[1], ***C. F. LEWALLEN**[1], C. R. LANDRY[2], I. KOLB[3], W. STOY[2], C. R. FOREST[1];
[1]Woodruff Sch. of Mechanical Engin., [2]Wallace H. Coulter Sch. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Janelia Res. Campus, Ashburn, AL

Abstract: Patch clamp recording remains the gold-standard technique for high-quality electrophysiological measurements of single cells in brain slices. Because of the many delicate steps required to perform this method, patch clamp recording is a high-skill, time-intensive

process. We have previously developed a robotic system, “the PatcherBot”, capable of performing unattended, sequential, multi-hour patch clamp experiments in brain slices, with a whole cell success rate of 50%. Yet connectomics and synaptic physiology studies instead require parallel, simultaneous, multiple pipette patch clamping. This has historically been performed manually, laboriously, and skillfully, with a single user operating multiple manipulators (up to 12) simultaneously. In order to automate this process, we have created a multi-pipette version of the PatcherBot that can move its pipettes in a coordinated fashion. Algorithms plan pipette routes to simultaneously avoid collisions (with each other) and efficiently patch synaptically connected cells embedded in tissue. Pipettes step over each other, in a process termed, “patch-walking,” such that pipettes retain whole cells while another pipette searches for cells connected to them. A preliminary computational model of the patch-walking strategy suggests that it can produce synaptic recordings up to 2-3 times the rate of manual experimenters. We will report progress and show a demonstration of this technology, representing a significant advance towards automated multi-pipette patch clamping.

Disclosures: M.C. Yip: None. **C.F. Lewallen:** None. **C.R. Landry:** None. **I. Kolb:** None. **W. Stoy:** None. **C.R. Forest:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.04/BB42

Topic: I.04. Physiological Methods

Title: Adaptive voltage protocols increase precision of voltage-gated ion channel measurements on high-throughput automated patch clamp platforms

Authors: S. WILLIAMS, J. KAMMONEN, *P. MITCHELL;
Integrated Biol., Charles River, Saffron Walden, United Kingdom

Abstract: Voltage-gated sodium (Na_V) channels are studied extensively due to their potential as targets for several indications, such as pain, epilepsy, cardiac and muscle paralysis. Some Na_V channel modulators show state-dependence and bind preferentially to the inactivated state of the channel. The potency of state-dependent compounds are known to vary depending on the percentage inactivation of the channels. To calculate accurate compound activity the precise value for the V_{half} of inactivation should be used for each cell. The adaptive protocol block for the Sophion Qube 384-well automated patch clamp platform has made it possible to separately define the voltage applied to each individual well for both the activation and inactivation of the channels, enabling the generation of more precise data for voltage-gated ion channels. The incorporation of the adaptive protocol did not change the performance of our $Na_V1.X$ assays compared to the standard protocol. For example, for Nav1.1 the mean percent current

inactivation was 54% in the adaptive protocol versus 44% in the standard protocol. The adaptive protocol significantly decreased the variability of the percent current inactivation: in the standard protocol experiment approximately 80% of the wells had percent current inactivation between 26-66%, whereas in the adaptive protocol experiment the percent current inactivation was between 47-61% for 80% of the wells. A range of known state-dependent compounds were tested as concentration-response curves against Nav1.X in both protocols, with compound potencies found to be similar between both protocols. However, the compound data at 10 µM was found to be much less variable in the adaptive protocol experiment, and in a high throughput screen this reduced variability should lead to increased confidence in the results. For Amitriptyline, the variation in percent inhibition at 10 µM was between 16-62% against Nav1.1, which may mean that in some cases the compound would not have been detected as a 'hit'. When using the adaptive voltage protocol, the variation in the percent inhibition was much smaller (52-62%). In summary, the new adaptive protocol enables increased control of the state that voltage-gated channels during an experiment on a 384-well high throughput automated patch clamp platform, which leads to reduced data variability and increased confidence in compound testing results.

Disclosures: S. Williams: None. **J. Kammonen:** None. **P. Mitchell:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

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Topic: I.04. Physiological Methods

Support: NIH Brain Initiative U01-MH106027- 01
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Edward Mallinckrodt, Jr. Foundation
Emory School of Medicine

Title: Automated electrophysiology of single cells in brain organoids

Authors: *C. R. LANDRY[1], M. C. YIP[2], C. F. LEWALLEN[2], Y. ZHOU[3], Z. WEN[4], C. R. FOREST[2];
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Abstract: Brain organoids allow neuroscientists to probe the development of human induced pluripotent stem cell (hiPSC) neurons in a three-dimensional environment. Organoid models have been used to link cell type development, migration, and structure to specific human genes or environmental factors. Significant interest exists in large scale single cell electrophysiology studies of brain organoids to quantify neural development and disease progression, as well as to enable pre-clinical drug screening. Current patch clamp methods are insufficient to handle the combinatorial expansion in recordings required to account for the size of these potential studies (e.g., variability, multiple time points, genetic variants). To this end, we have developed a robotic system that can perform sequential patch clamp recordings in brain organoids, cleaning and re-using the same pipette up to 100 times. The utility of this automated patch clamping system in the field of brain organoids will be demonstrated in several experiments: **1)** show the throughput capabilities of the robot by patching organoid-derived neuron cultures (12-15 cells per hour), **2)** demonstrate performance of robot in acute organoid slices, and **3)** present a method for deep patching (>100 µm from surface) in intact brain organoids, which may enable the study of synaptic function in organoids. This represents more than 10-fold improvement in the throughput and routine depth of patch clamp recordings in organoids, and thus may serve to dramatically accelerate research on organoids as model systems for human brain development.

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Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.06/BB44

Topic: I.04. Physiological Methods

Title: Neuronal activity in neurobasal A medium vs. ACSF: Which is best for whole-cell recordings in three-dimensional neural spheroids?

Authors: *M. R. ALKASLASI[1], B. B. THEYEL[2], J. L. SEVETSON[3,1,4], D. HOFFMAN-KIM[3,4,5,6], B. W. CONNORS[1];
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Abstract: In comparison to traditional two-dimensional culture, three-dimensional neural spheroid models offer a way to study cellular mechanisms *in vitro* while recapitulating key elements of *in vivo* cytoarchitecture. Neural spheroids are currently used as models for studies of various neurological disorders and injuries, including autism spectrum disorder and stroke (Amin and Paşca, *Neuron*, 2018). Neural spheroids enable control over the cellular composition of the

tissue and alterations in developmental conditions, manipulations not easily achieved in whole animals. As a model that bridges the gap between two-dimensional cultures and tissue obtained from animal models, it is important to systematically determine what recording conditions yield the most robust, *in vivo*-like network activity while maintaining tissue health. It has been previously established that DMEM/F12 basal and Neurobasal media impair neuronal activity in culture compared to recordings performed in artificial cerebral spinal fluid (ACSF) (Bardy et al., *Proc Natl Acad Sci USA*, 2015). To our knowledge, however, no similar analysis of recording media has been undertaken in neural spheroids. In this study, we evaluate cellular activity in three-dimensional neural spheroids, comparing two extracellular recording solutions for whole-cell patch clamp electrophysiology: Neurobasal-A-based cell culture medium and ACSF. Primary rat cortical cells were isolated postnatally (P0-P3) and allowed to self-assemble into three-dimensional spheroids at 8000 cells/spheroid as per a previously established model (Dingle et al., *Tissue Eng Part C Methods*, 2015). Electrophysiological recordings were collected at day 10-14 at room temperature from spheroids initially in a Neurobasal A-based cell culture medium, and the medium was gradually (1ml/min) replaced with oxygenated ACSF. We found that the resting membrane potential of neurons in Neurobasal A medium was consistently depolarized relative to neurons in ACSF. Neurons required significantly more current to reach action potential threshold and exhibited a significantly lower peak firing rate when in Neurobasal A medium, relative to ACSF. Developing a greater mechanistic understanding of the electrophysiological effects different recording media have on the electrophysiology in three-dimensional, self-assembled, scaffold-free neural spheroids will allow us to identify the ideal conditions for studying their neuronal activity.

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Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

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Program #/Poster #: 091.07/BB45

Topic: I.04. Physiological Methods

Support: BIH grant CRG 2b T1
DFG grant SFB/TRR 186, A4

Title: Human induced neuronal autaptic cultures to study synaptic function and neuronal morphology

Authors: *P. FENSKE, M. K. GRAUEL, M. M. BROCKMANN, A. L. DORRN, T. TRIMBUCH, C. ROSENMUND;
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Abstract: Human induced neurons (iNs) derived from induced pluripotent stem cells (iPSCs) hold great potential for understanding human neurological disorders and their underlying mechanisms. So far, protocols for differentiation of stem cells to human neurons with functional synapses require months of culturing. Furthermore, since available protocols rely on conventional mass culture, where many neurons are plated together, the resulting preparation allows only for limited access to parameters that define synaptic input and output function and morphology of individual neurons due to the complex innervation patterns and weak connectivity between individual pairs of neurons. We have therefore developed a two-phase culturing approach to obtain for the first-time cultures of single-human neurons that form self-synapsing autaptic connections. We show that these human autaptic neurons, like their rodent counterparts, provide a standardized, high fidelity assessment of human synapse formation and synaptic transmission. Our protocol to generate glutamatergic autaptic cultures from iPSC-derived iNs, is based on neurogenin-2 (Ngn2) expression. Electrophysiological and immunocytochemical analyses show that our method efficiently generates mature autaptic iNs, with normal synapse densities and robust spontaneous and action potential-driven glutamatergic release. We propose that the human iN autaptic culture system provides a versatile platform allowing for quantitative and integrative assessment of morphophysiological parameters underlying human synaptic transmission. Moreover, we believe that the approach will therefore have a great impact for future investigations of human synaptic function as well as synaptopathies underlying human neurological diseases.

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Poster

091. Techniques: Cellular Electrophysiology

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Minerva

Title: A novel approach for single trial AC measurement of membrane conductance under current clamp

Authors: A. PARABUCKI, M. SOKOLETSKY, H. FAMINI, Y. KATZ, ***I. LAMPL**; Weizmann Inst. Sci., Rehovot, Israel

Abstract: Intracellular recordings under current clamp allow investigating the natural time evolution of membrane potential (Vm) due to synaptic and voltage-dependent conductances. On the other hand, voltage clamp recordings enable precise measurements of underlying conductances, but not without the price of altering the time evolution of Vm, due to the clamping. Here we developed a new method to record Vm of neurons under current clamp while simultaneously measuring their underlying conductance with a high temporal resolution and in single trials.
Based on a simple alternating current (AC) analysis of RC circuits, we found that due to the series resistance of the intracellular electrode, the total input impedance of a model cell and the recording electrode at high frequencies (>> 1/RC) grows linearly with increasing membrane conductance. This impedance property stems from the short cable structure of the electrode-cell circuit and thus was not observed when the electrode resistance was set to zero. Accordingly, we expected that by injecting a small sinusoidal current at a high frequency (80-500 Hz), well above the spectral content of subthreshold activities, we will be able to track changes in the cell's conductance. Indeed, this was confirmed in a simulation of a point neuron, recorded via a simulated pipette, receiving synaptic inputs and additional noise. Finally, we experimentally tested our method by recording from cortical neurons under current clamp (without bridge balance) while injecting high frequency sinusoidal current (Vm response of 2-5 mV in amplitude) in brain slices and anesthetized GAD-ChR2 mice. While light illumination reduced the input resistance of excitatory neurons due to shunting inhibition (confirmed from injection of current pulses), it actually increased their impedance for the high frequency injected current, as predicted from our theoretical analysis. Furthermore, these measurements were obtained in single trials and by using a band-stop filter we were able to reconstruct the actual Vm of the cells. We present the limitations of this approach, such as its sensitivity and accuracy when studying underlying changes in membrane conductance under current clamp. Our method can be potentially used when studying the underlying conductance changes of cells' Vm under current clamp, keeping voltage-dependent mechanisms unaffected. Due to the high frequency of the injected current, our approach allows studying fast (millisecond) changes in membrane conductivity during intact Vm activity. This approach is of potential use when testing the effects of drugs on synaptic or intrinsic conductances both *in-vitro* and *in-vivo*.

Disclosures: A. Parabucki: None. **M. Sokoletsky:** None. **H. Famini:** None. **Y. Katz:** None. **I. Lampl:** None.

Poster

091. Techniques: Cellular Electrophysiology

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Program #/Poster #: 091.09/BB47

Topic: I.04. Physiological Methods

Support: MSCA grant #793482
ERC grant #694539

Title: Ultrastructural and physiological aspects of synapses in acute brain slices prepared at cold and physiological temperatures

Authors: *K. EGUCHI, P. KOPPENSTEINER, R. SHIGEMOTO;
Mol. Neurosci., IST Austria, Klosterneuburg, Austria

Abstract: Acute brain slice preparation has been developed as a powerful experimental model for investigating the structural and functional characteristics of synaptic connectivity of neuronal circuits in brain. The acute slice preparation is readily accessible for electrophysiological and optical recordings and retains the cytoarchitecture and synaptic circuits *in vivo* except for the long-range projections. In general, to prepare acute slices, the dissected brain from an animal is quickly immersed into the ice-cold cutting solution, and then sliced at 2-300 μm intervals by a microtome at ice-cold temperature to avoid the neuronal excitotoxicity caused by mechanical injury during cutting. The acute slice preparation at ice-cold, however, causes molecular and architectural changes of cellular components. Chilling of hippocampal slices induces disassembly of microtubules and eliminates dendritic spines and cytosolic glycogen granules. Re-warming of the hippocampal slices revives microtubule structures and glycogen granules, but excessively reconstructs the dendritic spines resulting in higher density of synapses than that in intact brain (Kirov et al., 2004).
Recently, a method of acute slice preparation at physiological temperature (PT) has been developed to improve quality of cerebellar slices in aged rodents. In the warm-cutting cerebellar slices, Purkinje cells (PCs) survived better without altered intrinsic electrophysiological features of the cells (Huang and Uusisaari, 2013). However, it hasn't examined whether the synaptic features are better preserved in the warm-cut acute slices compared to the cold-cut slices.
In this study, we investigated which method would provide us with brain slices closer to the intact brains using electron microscopic and electrophysiological analysis. To evaluate the synaptic properties in acute cerebellar slices of mice prepared at ice-cold and PT, we compared synaptic vesicle distribution in presynaptic boutons, protein distribution in post- and presynaptic sites and electrophysiological properties of neurotransmission. Our results indicate that the warm-cut slice method provides us brain slices almost similar to intact brains compared to the cold-cut method and also give us some additional advantages such as saving time for experiments.

Disclosures: K. Eguchi: None. **P. Koppensteiner:** None. **R. Shigemoto:** None.

Poster

091. Techniques: Cellular Electrophysiology

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Program #/Poster #: 091.10/BB48

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Strategic Advancement of Multi-Purpose Ultra-Human Robot and Artificial Intelligence Technologies program from NEDO
Toyota Physical & Chemical Research Institute Scholars

Title: Fine marking method of metal microelectrode for the electrophysiological recording *in vivo*

Authors: *K. KOIDA[1], H. SAWAHATA[2,1], R. NUMANO[1], T. KAWANO[1], T. HARA[1];
[1]Toyohashi Univ. of Technol., Toyohashi, Japan; [2]Natl. Inst. of Technology, Ibaraki Col., Ibaraki, Japan

Abstract: Metal microelectrodes have been widely used in the electrophysiological experiments, however spatial reconstruction of recording site have limit in its resolution compared to cellular scale. Various marking methods have been developed however there still exists problems not only its resolution but also a tissue damage due to marking, and sustainability of the marking for chronic experiments.
Here we introduce a novel marking method by applying plating and electrolysis of the electrode tip to enable precise localization of recording site. Double-layer plating is applied to the tip of the metal electrode, the plating of superficial layer is made of a noble metal, and the plating of deep layer, or connecting layer, is made of a relatively base metal. When an electric current is passed through the metal electrode, the metal of the connecting layer is selectively dissolved and broken, and the noble metal plating at the tip is detached from the electrode. Detached tip could become a very small marking of the electrode site.
We demonstrated the method by using a platinum electrode plated by gold (Au) as a connecting layer and platinum (Pt) as a superficial layer. After inserting the electrode into anesthetized mouse cerebral cortex, positive electrode current less than micro ampere successfully dissociated the tip, and resulted the marking point at the site. Marking was visible by conventional microscope without any staining. We also succeeded similar marking after single unit recording *in vivo* by double plating of Au-Pt to tungsten electrodes. Validity of the marking site was confirmed by visualizing the electrode insertion path with fluorescent dye. Furthermore, no serious damage of the tissue around the marking site was observed. The marking size was about 5~30 μm in width which was smaller than the existing methods. It will be necessary to

investigate the applicability of electrodes not only in single electrode but also in high-performance multi-electrodes.

Disclosures: K. Koida: None. **H. Sawahata:** None. **R. Numano:** None. **T. Kawano:** None. **T. Hara:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.11/BB49

Topic: I.04. Physiological Methods

Support: NIH Grant MH113341

Title: Ionic mechanisms underlying firing pattern of dopaminergic neurons during a noxious stimulus

Authors: *J. M. PERKINS, A. KULKARNI, C. PALADINI;
Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Midbrain dopamine neurons are strongly implicated in reward and aversion. Aversive stimuli are encoded by the firing pattern of dopamine neurons, which governs when dopamine is released at target regions. Separate subpopulations of dopamine neurons show either an increase or a decrease in firing rate in response to aversive stimuli. A response to an aversive stimulus is generated by integrating activity from multiple afferents, and changes in dopamine neuron firing activity are the result of dynamic changes in synaptic input. Using intracellular *in vivo* recordings, we are able to differentiate the excitatory and inhibitory subthreshold ionic conductances that elicit either an increase or decrease in dopamine firing activity. We investigated the ionic mechanism underlying dopamine neuron pause or excitation during a foot pinch or shock. A pause in firing during a tail pinch could be due to either a decrease in excitation or an increase in inhibition. An increase in firing rate could be due to either an increase in excitation or a decrease in inhibition. Preliminary results suggest that a large hyperpolarization is present during a pause in firing following a noxious stimulus, and the hyperpolarization is driven by an increase in total ionic conductance. An increase in firing activity during a noxious stimulus is the result of a depolarization which is also driven by an increase in total ionic conductance. Determining the underlying ionic mechanisms mediating changes in firing pattern will provide insight into the afferents that elicit a response during a tail pinch.

Disclosures: J.M. Perkins: None. **A. Kulkarni:** None. **C. Paladini:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.12/BB50

Topic: I.04. Physiological Methods

Support: R21 MH113341

Title: Subthreshold synaptic dynamics in midbrain dopamine neurons and their role in driving spiking activity

Authors: *A. S. KULKARNI[1], K. OTOMO[2], J. PERKINS[1], S. STOJANOVIC[3], J. ROEPER[3], C. PALADINI[1];
[1]Biol., Univ. of Texas At San Antonio, San Antonio, TX; [2]Inst. of Neurophysiol., [3]Goethe Univ. Frankfurt, Frankfurt Am Main, Germany

Abstract: Midbrain dopamine neurons regulate critical aspects of reward and motivation related behavior. In order to understand how dopamine neurons transform their diverse inputs into reward related signals, it is important to characterize their activity in spiking (across bursting and pace-making modes) and subthreshold domains. More importantly, an understanding of what drives the cell to fire rapidly or slowly and what drives the cell to burst and pause, can give us crucial functional insights. The best, albeit laborious and high-skill, technique available to attack these questions is *in vivo* whole-cell recording. We have developed a method to consistently obtain *in vivo* whole-cell recordings from dopamine neurons. We also fill our cells after the recording and determine the cell location with reference to a standardized brain atlas.
We have an extensive data set with more than 100 cells that encompasses wide heterogeneity in firing rate, synaptic input, spike waveform, cell location, and a variety of other parameters of interest. We developed custom algorithms to excise spikes from our traces and then removed additional spike-adjacent portions of the trace to ensure that the remaining portion primarily consists of synaptic fluctuations and non-spike dynamics. We used various metrics, like root-mean-squared level of the highpass-filtered inter-spike trace (IST) and synaptic event detections, to quantify the amount and direction of synaptic input being received during a particular IST. We investigated the relationship between synaptic input and firing rate within and across cells. Preliminary analysis suggests that widely different levels of synaptic input can result in highly similar firing rates. We also found that across cells, higher levels of synaptic input, drive higher variability in pacemaker firing. Additionally, we used ISTs to characterize sub-threshold signatures of bursts and pauses and establish sub-threshold criteria to detect them. We compare our results to those obtained by well-established heuristic and statistical methods for burst and pause detection. Finally, we investigated correlations between various quantities calculated for a cell and its location within the standardized brain atlas.

Disclosures: **A.S. Kulkarni:** None. **K. Otomo:** None. **J. Perkins:** None. **S. Stojanovic:** None. **J. Roeper:** None. **C. Paladini:** None.

Poster

091. Techniques: Cellular Electrophysiology

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 091.13/BB51

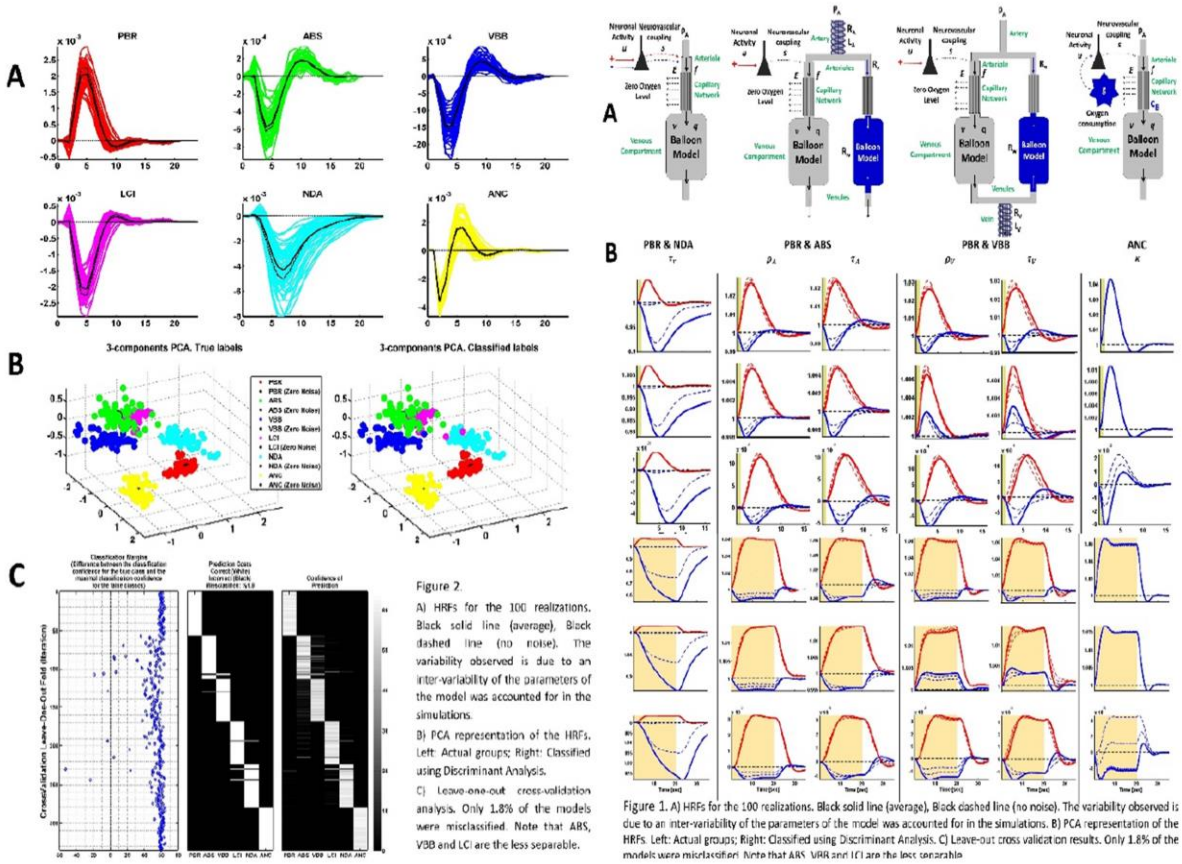
Topic: I.04. Physiological Methods

Support: NIH (1R56NS094784-01A1)

Title: Mechanisms of negative BOLD responses

Authors: ***J. J. RIERA**, P. VALDES HERNANDEZ, A. MOSHKFOROUSH;
Florida Intl. Univ., Miami, FL

Abstract: Alongside positive BOLD responses (**PBR**), negative ones (NBR) appear in interictal epileptiform discharges (IEDs). Contrary to PBR, there is no consensus about the NBR mechanisms. We identify **five** of these and propose biophysical models to describe them: 1) arterial blood stealing (**ABS**)(Harel, et al. 2002), 2) vein blood backpressure (**VBB**)(Bandettini, et al. 2012), 3) lateral/callosal inhibition (**LCI**)(Shmuel, et al. 2006), 4) neuronal disruption of activity (**NDA**), with a slow recovery, of resting state networks (Laufs, et al. 2007) or 5) altered neurometabolic couplings (**ANC**) (Song et al. 2016). We show that, under realistic physiological/observation noise and model parameter variability, the HRFs estimated from BOLD data of these different mechanisms are distinguishable. Schematics of the models are depicted in Figure 1A. Balloon models describe the PBR, LCI and NDA mechanisms, with the incorporation of a baseline neuronal activity that is decreased by inhibitory inputs (and delayed in NDA) driven by PBR exhibiting areas. We model ABS and VBB as two windkessels sharing the same feeding artery and the same output vein, respectively, accounting for blood resistance and viscosity. We model ANC using the oxygen to transport model, but increasing the neurometabolic coupling gains. To obtain estimates of the HRF of each of the abovementioned mechanisms we simulated 100 realizations of 40-min BOLD signals after IEDs. Each model—in a state-space form—comprises a set of SDE equations and the BOLD observation equation. The systems were integrated using the Local-Linearization method. These BOLD signals were fitted to an ARX model to obtain the HRF temporal profiles that were classified using discriminant analysis of the first 3 PCAs. Fig. 1 shows BOLD responses predicted by the models and their sensitivity to a set of the relevant parameters. The 3D representation and their classification of the ARX-based PCA components is shown in the figure. We propose 5 NBR mechanisms. We demonstrate they can be distinguished using BOLD signals.



Disclosures: **J.J. Riera:** None. **P. Valdes Hernandez:** None. **A. Moshkforoush:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.01/BB52

Topic: I.07. Data Analysis and Statistics

Title: Automated parameter search for fmri preprocessing pipeline quality control using functional connectivity matrix clustering

Authors: ***M. KOLLADA**, H. GONZALEZ, Y. LIU, M. S. MELLEM, P. AHAMMAD; Blackthorn Therapeut., San Francisco, CA

Abstract: Background: Quality Control (QC) of fMRI preprocessing has become a bottleneck to analyzing large-scale fMRI datasets. The need for human-in-the-loop iterations through preprocessing parameters to manually identify correctly preprocessed output images requires

substantial time from expert reviewers. We propose a method that, for each subject, automatically searches a large set of preprocessing parameters to predict those that will pass visual QC. **Methods:** We preprocessed MRI data from two publicly available MRI datasets, CNP LA5c (N=251) and EMBARC (N=330), using 72 different parameter sets. This was enabled by our ability to perform massively parallel fMRI preprocessing with our in-house developed, cloud-enabled pipeline based on AFNI (http://afni.nimh.nih.gov/afni). These 72 parameter sets were created by varying four different parameters that commonly need human-led optimization - two from the structural-functional alignment step and two from the skullstripping step. For each of the 72 pipeline outputs per subject, we generated Functional Connectivity (FC) matrices and grouped them by similarity into clusters based on the Frobenius norm of the pairwise difference between the matrices. The similarity threshold used to group the matrices was set as the smallest value such that a dominant, stable cluster was found, indicated by the size ratio between the two largest clusters being at least 2 to 1. The centroid of the largest cluster of parameters for each subject was selected as our prediction to pass QC and the algorithm-generated predictions were validated using visual QC from expert reviewers. **Results:** We compared our automatic parameter prediction method against a control method of using a single, expert-selected set of parameters for subjects in two independent datasets. The control method was chosen as an estimate of results given the same amount of reviewer effort without our prediction method. Using 50 randomly selected subjects from each dataset, our automatic parameter prediction method had 92% of subjects pass visual QC for CNP and 80% for EMBARC, while the control method passed only 62% of subjects for CNP and 70% for EMBARC. **Conclusion:** We developed an automated search method for selecting the optimal fMRI preprocessing pipeline parameters that has been validated on two independent datasets. Our method allows us to generate parameter set recommendations for each subject, therefore dramatically reducing the turnaround time and effort required of an expert reviewer to fully QC a dataset. It results in a novel, efficient, and effective method to perform QC of preprocessed fMRI images.

Disclosures: **M. Kollada:** A. Employment/Salary (full or part-time):; Blackthorn Therapeutics. **H. Gonzalez:** A. Employment/Salary (full or part-time):; Blackthorn Therapeutics. **Y. Liu:** A. Employment/Salary (full or part-time):; Blackthorn Therapeutics. **M.S. Mellem:** A. Employment/Salary (full or part-time):; Blackthorn Therapeutics. **P. Ahammad:** A. Employment/Salary (full or part-time):; Blackthorn Therapeutics.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.02/BB53

Topic: I.07. Data Analysis and Statistics

Title: The network analysis using synchronization likelihood and cross-correlation for electroencephalogram during facial expressions

Authors: *A. WATANABE, T. YAMAZAKI;
Kyushu Inst. of Technol., Iizuka, Japan

Abstract: Objective: The brain is controlling many activities of the body and has some important roles in emotions. Especially, 'smiling' is an example of physical activity that causes many good effects on the body. We assumed the relationship between a treatment efficiency and emotions could be identified. To get an estimation of the activated brain areas, we proposed a method to construct and quantify brain functional connectivity networks (BFCNs). These show which pairs of electrodes are highly synchronized, determined by synchronization likelihood (SL) that is calculated from electroencephalograms (EEGs). The method reveals the tendency of the activation in different brain areas during various facial expressions, such as smiling, angry and sad. From our previous study, the activated area seems to follow the facial muscle movement in gamma band. However, the right side of the brain were highly activated by smiling. Then, the method must be developed and be more valid to detect the activated brain area. **Method**: Our subjects are all Japanese females and males, nineteen to twenty-six years old. We will gather the data from around twenty-five subjects in the further study. EEGs are recorded with 19 channels on the basis of international 10-20 system. We applied the method we proposed to their EEGs measured during various facial expressions. Then, we also calculated the cross-correlation between each channel pair and constructed BFCNs by the same manner with SL based BFCNs. We focused on the peaks of those variables to construct BFCNs. We compare both BFCNs and quantify the efficiency of the methods. **Results**: BFCNs are plotted in the axis of those Rightness vs Frontness. For now, there is an opposite tendency in SL based and cross-correlation based BFCNs during biased facial muscle movements. Almost the activated areas part to right and left. **Conclusions**: The facial expressions, especially smiling, can be differentiated by our method to estimate the activated brain area. The tendency from SL based BFCN is supported by comparing with the result of cross-correlation analysis.

Disclosures: A. Watanabe: None. **T. Yamazaki:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.03/BB54

Topic: I.07. Data Analysis and Statistics

Support: Veritas Fund

Title: Total electrical noise in human intracranial microwire recordings and its simulation

Authors: *P. N. STEINMETZ;
NeurTex Brain Res. Inst., Dallas, TX

Abstract: Given the small electrical signals (~35 microvolts) present in human intracranial microwire recording, many sources of electrical noise may interfere with the ability to accurately isolate the activity of single neurons. These include both extrinsic sources of noise, such as nearby electrical circuits, but also other nearby neurons whose action potentials (APs) cannot be reliably isolated.
To better understand how these noise sources limit the detectability of single neuron signals, I examined the recorded levels of noise in a variety of human intracranial microwire recording systems at different institutions. The power spectral densities (PSDs) of the raw unfiltered recordings were computed and displayed on both linear and log-log scales (Bode magnitude plot).
These recordings show substantial levels of powerline interference and other likely demodulation interference often exist in these recordings. They also show substantial variation in all types of noise between both recording sites and particular channels in individual patients. Typically there is ~5 microVolts (rms) noise in the passband (300-3000 Hz) used for spike sorting.
There are also substantial amounts of noise power within frequency ranges where there is a difference in power between different recorded waveform shapes (100-600, 1800-2500 Hz). Noise in these frequency ranges are critical determinants of the ability to separate the activity of single neurons from one another.
Given the complex nature of the noise sources and their impact of spike sorting performance, an analytic solution to the performance of AP waveform detection and sorting is not feasible. Thus I designed a 6 pole and 6 zero linear filter which can be used to simulate colored noise which mimics the baseline effects of actual total noise sources in these recordings. It differs from prior simulations of noise in this type of recording by including empirically observed noise sources and can simulate any length of recording. It may also be readily combined with simulated powerline or other forms of interference. This simulated noise can be used to study the effects of all noise soures on detection of AP waveforms as well as firing rate changes in the human brain.

Disclosures: P.N. Steinmetz: None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.04/BB55

Topic: I.07. Data Analysis and Statistics

Support: NIGMS GM095653
NIH T32 GM089626-09

Stanford University School of Medicine Department of Medicine, Translational Medicine Award

Title: Nonlinear dynamics analyses of EEG signals capture brain states at different levels of consciousness

Authors: *S. L. EAGLEMAN[1], D. CHANDER[2], C. REYNOLDS[5], N. OUELLETTE[3], B. MACIVER[4];
[2]Med., [3]Civil and Envrn. Engin., [4]Anesthesia, [1]Stanford Univ., Stanford, CA; [5]Neurol., Oregon Hlth. Sci. Univ., Portland, OR

Abstract: Anesthetic transition periods around loss and recovery of consciousness are not always captured by behavioral assessment or standard linear analyses of EEG. Propofol is one of the most widely used anesthetics for routine surgical anesthesia. Propofol administration alone produces EEG spectral characteristics similar to most hypnotics; however, inter-individual variations often make spectral measures inconsistent. Complexity measures of EEG signals could offer universal measures to better capture anesthetic depth as brain activity exhibits nonlinear behavior at several scales. Patients undergoing propofol general anesthesia for various surgical procedures were identified as having changes in states of consciousness by the loss and recovery of response to verbal commands after induction and upon recovery from anesthesia, respectively. We demonstrate that complexity measures, derived from nonlinear dynamics techniques, and captured in attractors, distinguish such states reliably and with sensitivity. Notably, nonlinear dynamics analyses showed more significant differences between consciousness states than most spectral measures. Additionally, we found these measures are dependent on analysis features and show tight correlation with spectral measures during consciousness transition states. Thus, these measures could provide a means for reliably capturing depth of consciousness based on subtle EEG changes at the beginning and end of anesthesia administration. In addition, complexity is able to more fully describe how different these brain states are. For example, the attractors generated through time-delayed embeddings during anesthesia exhibit significantly different shapes, which have implications for network connectivity and information processing in the brain. This work supports existing theories on neural correlates of consciousness showing a diminished information carrying capacity of the brain during decreasing levels of consciousness.

Disclosures: S.L. Eagleman: None. **D. Chander:** None. **C. Reynolds:** None. **N. Ouellette:** None. **B. MacIver:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.05/BB56

Topic: I.07. Data Analysis and Statistics

Title: Generalization performance improvement method using automatic feature selection in pathological voice detection

Authors: *K. SUZUKI[1], S. SHINOHARA[2], N. MANOME[1], M. HIGUCHI[3], Y. OMIYA[5], S. MITSUYOSHI[4];
[1]SoftBank Robotics Corp./The Univ. of Tokyo, Tokyo, Japan; [2]Dept. of Verbal Analysis of Pathophysiology Grad. Sch. of Med., [3]The Univ. of Tokyo, Tokyo, Japan; [4]Verbal Analysis of Pathophysiology, The Univ. of Tokyo, Yokohama-Shi, Japan; [5]PST Inc., Yokohama-Shi, Naka-Ku, Japan

Abstract: Pathological voice detection with smart devices is essential for providing low-cost and high-quality health care to every people. To build machine learning-based prediction models, extracting many types of acoustic features from voice samples improves the ability of accurate detection, but containing too many types of features may cause the overfitting. Thus, it is required to select features which are essential to detect pathological conditions. Conventionally, feature selection has been operated by manual with the domain knowledge of diseases. We propose to select features automatically by using Boruta, which is random forest-based feature selection algorithm. We suppose learning with automatically selected features improves the generalization performance of pathological voice detection. To validate the hypothesis, we conduct the following experiment. We used pathological voice samples from two datasets, Saarbrucken Voice database (SVD) and Massachusetts Eye and Ear Infirmary database (MEEI). These databases contain both voice samples from normal people and disease people, and are different in terms of the recording environment, the distribution of age, gender, etc. Thus, we evaluate the generalization performance by using these databases. From SVD, we choose healthy voice samples and voice samples with 3 diseases (Cyste, Polyp, Paralysis) by references to the research of Alhussein et.al. From MEEI, we use the subset which is proposed by the research of Godino-Llorente et.al. and widely used by some researches. In both datasets, we labeled normal voice samples as 0 and pathological voice samples as 1. For machine learning, we extracted 6,373 voice features from these datasets using openSmile(ComParE13.conf) . Then we train a model using voice samples from SVD and test the model using voice samples from MEEI. To learn the training dataset, we use LightGBM. For Boruta, we set the number of the iteration as 40. During feature selection by Boruta, the number of features decreases from 6373 to 189. The numerical experiment shows that accuracy without Boruta is up to 53.5 % and accuracy with Boruta is 57.5 %. This result describes feature selection through Boruta contributes the improvement of generalization performance.

Disclosures: K. Suzuki: None. **S. Shinohara:** None. **N. Manome:** None. **M. Higuchi:** None. **Y. Omiya:** None. **S. Mitsuyoshi:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.06/BB57

Topic: I.07. Data Analysis and Statistics

Title: Parkinson's disease detection from a small amount of data based on self-organizing maps

Authors: *N. MANOME[1], S. SHINOHARA[2], K. SUZUKI[1], Y. OMIYA[3], M. HIGUCHI[2], S. MITSUYOSHI[2];
[1]SoftBank Robotics Corp./The Univ. of Tokyo, Tokyo, Japan; [2]The Univ. of Tokyo, Tokyo, Japan; [3]PST Inc., Yokohama-Shi, Naka-Ku, Japan

Abstract: In recent years, pathological analyses that use abundant calculation resources have been actively carried out. However, techniques for analyzing diseases at reduced costs are desired, and we are engaged in the development of techniques that will allow us to analyze diseases using a handy device, like a smartphone. Furthermore, in the medical field, there are many cases where learning data for pathological analyses can be obtained sequentially. Thus, this study used self-organizing maps (SOMs), an online machine learning method, to test whether the technique can be used to discriminate between patients with Parkinson's disease and healthy people, from limited amounts of learning data. SOMs are artificial neural networks that were invented by Kohonen and allow high speed calculations on handy devices, like a smartphone. The data set that was used in this study consists of 756 items of speech data that were obtained from 252 participants (188 patients with Parkinson's disease, 64 healthy individuals) at Istanbul University. These speech data were recordings of sustained phonations of the vowel /a/, uttered three times by each participant. The experimental procedure of this study is as follows: First, the data were divided into two kinds, learning data and test data. Then, learning data were randomly shuffled, extracted, and learned one-by-one using SOMs. In each stage of learning, the accuracy was calculated based on test data. For learning, 753 acoustic features that included mel-frequency cepstral coefficients, which were extracted from each speech datum, were used. In this study, to demonstrate the usefulness of SOMs, the experimental procedure was repeated 1000 times, and the mean accuracy was calculated. SOM parameters were set as follows: size of competitive layers, 10×10; number of neurons, 100; initial learning rate, 0.3; initial neighborhood radius, 5; minimum neighborhood radius, 2. Our experiment revealed that the accuracy was 0.748 when all of the learning data were used, 0.743 when 50% of the learning data were used, and 0.734 when 20 % of the learning data were used. These results suggest that the use of SOMs may allow us to discriminate between patients with Parkinson's disease and healthy individuals through the use of smaller amounts of learning data at a precision that is not very different from the precision that is attained when all of the learning data were used. In the

future, we will identify appropriate parameter settings for SOMs and develop methods that will allow us to analyze diseases with ease, using even smaller amounts of learning data.

Disclosures: **N. Manome:** None. **S. Shinohara:** None. **K. Suzuki:** None. **Y. Omiya:** None. **M. Higuchi:** None. **S. Mitsuyoshi:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.07/BB58

Topic: I.07. Data Analysis and Statistics

Title: Language mapping using high-density EEG: A large-scale network connectivity analysis

Authors: *V. YOUSSOFZADEH[1], F. SALAMI[2], A. BABAJANI-FEREMI[3];
[1]Med. Col. of Wisconsin, Milwaukee, WI; [2]Pediatrics, [3]Dept. of Pediatrics, The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: High-density electroencephalography (hdEEG) is an emerging brain imaging technique that offers high temporal resolution and wide coverage, thereby facilitates studying source space interactions on a behaviorally relevant time-scale. Language mapping in hdEEG is currently limited by a number of methodological issues, among which the difficulty in obtaining accurate localization patterns and laterality indices. We propose a large-scale network connectivity analysis using hdEEG to provide robust maps of the receptive and expressive language processes, comparable to maps provided by functional MRI (fMRI). hdEEG and fMRI data were collected from 22 healthy adults (9 female, 20−37 yrs) during two language experiments, word-recognition 'receptive' task (WRT) and verb generation 'expressive' task (VGT). For hdEEG language mapping, a head model was computed based on a boundary element method. Voxel-level sources were estimated using a spatial beamformer in a time-frequency range of 400−700ms and 3−30Hz, as indicated significant event-related desynchronization effects. Connectivity was computed using phase locking value. Brain hubs were characterized using an eigenvector centrality (EC) graph measure. fMRI data were analyzed using a classic general linear model approach. hdEEG network hubs and fMRI activations were compared within 20 atlas-driven frontotemporal regions. hdEEG network analysis of responses associated with WRT and VGT showed predominant hubs in left superior temporal gyrus (STG; EC=0.84) and left inferior frontal gyrus (IFG; *pars triangularis*, EC=0.91 and *pars opercularis*, EC=0.84), respectively. Consistently, hdEEG laterality index (LI) of WRT and VGT showed strong asymmetry in temporal (LI=0.65±0.23) and frontal regions (LI=0.54±0.45), respectively. Unlike hdEEG, fMRI analysis of WRT showed activations within bilateral STG but favored a right hemisphere dominance (right STG, LI=-0.21±0.35). fMRI analysis of VGT responses, in agreement with hdEEG, localized IFG regions (pars triangularis

and pars opercularis, LI=0.56±0.13). This study demonstrates the suitability of hdEEG network analysis in identifying brain hubs associated with language processing function. hdEEG localization and lateralization analyses supported a consistent left-hemispheric dominance during WRT and VGT and showed an overall good concordance with fMRI findings. hdEEG has the potential to be utilized as a fast and low-cost modality for presurgical language mapping in a clinical population.

Disclosures: V. Youssofzadeh: None. **F. Salami:** None. **A. Babajani-Feremi:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.08/BB59

Topic: I.07. Data Analysis and Statistics

Title: Characterization of functional hierarchical organization of human brain based on intrinsic connectivity networks

Authors: C. CHU[1], L. FAN[1], Y. LIU[1], D. WU[1], J. SUI[1], S. B. EICKHOFF[2], ***T. JIANG**[1]; [1]Inst. of Automation, Chinese Acad. of Sci., Beijing, China; [2]Inst. for Systems Neuroscience, Heinrich-Heine Univ., Düsseldorf, Germany

Abstract: Spontaneous fluctuations underlying brain activity can reflect the intrinsic organization of the system, which could be described as the functional brain networks. Using networks or connectomics to measure the pattern of brain activities has emerged as a new avenue to explore the large-scale functional properties of human brain. Functional diversity of different brain region is an important and intriguing functional property of human brain, which would be a hint for the potential cortical hierarchy. So, we here tried to propose a meaningful way to characterize the distributed functional diversity of human brain regions based on the network perspective. We hypothesized that the patterns of participating in different functional networks were related with the functional diversity of particular brain regions. Independent component analysis (ICA) was adopted to detect the intrinsic connectivity networks (ICNs) based on the resting-state functional MRI data. An index of functional diversity (FunDi) was proposed to quantitatively characterize the extent of anisotropic distribution related with participation of various ICNs. Fig. 1 provided an overview of these analytic steps. We found that FunDi continuously varied across the brain, for example, the primary motor cortex showing a low FunDi value and the inferior parietal lobule having a higher value. Further, we validated the proposed index by comparing the well-demonstrated homotopic regions between left and right hemisphere where the language-related Broca's area popped out showing lower FunDi value in the left hemisphere. The pattern of FunDi value across the whole brain, higher in association cortex and lower in primary cortex, reminded us that the proposed index might be able to work

as a new approach to quantitatively characterize the functional hierarchical organization of human brain, which could be utilized to detect the functional development across different growth stage or to provide a starting point for the large-scale brain network simulation in the future.

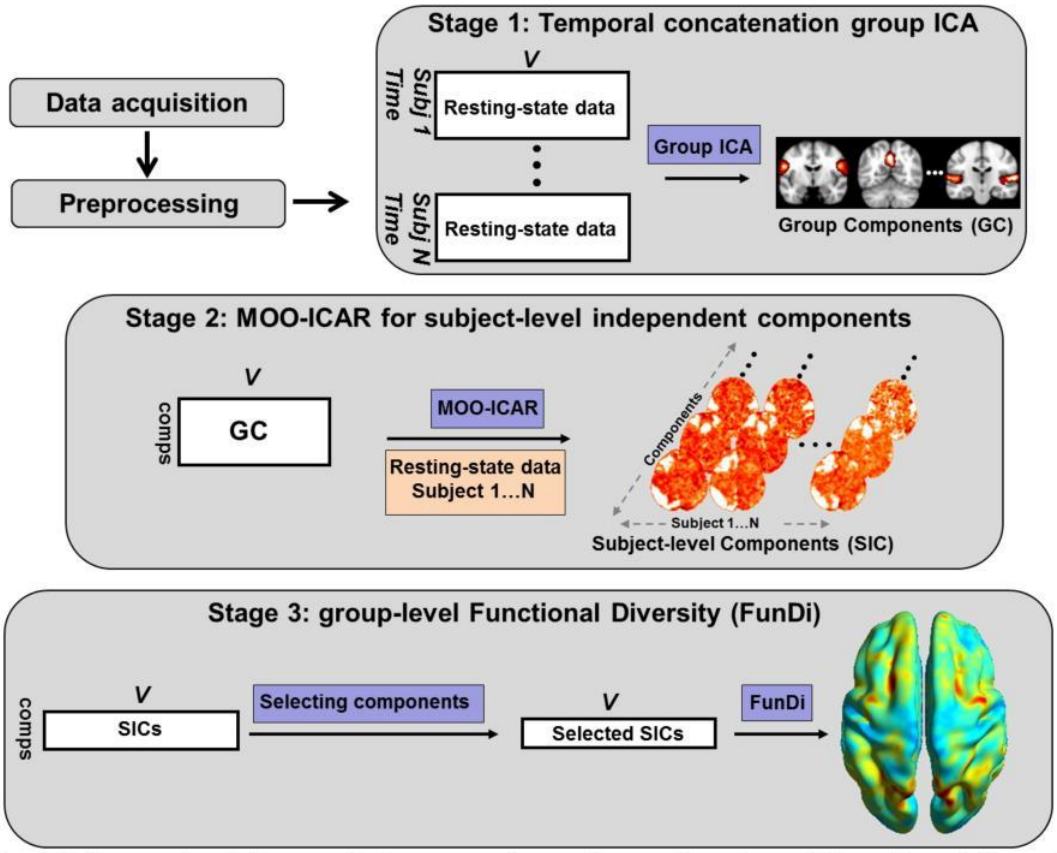


Fig.1 Schematic of the analytic steps about the estimation of functional diversity

Disclosures: **C. Chu:** None. **L. Fan:** None. **Y. Liu:** None. **D. Wu:** None. **S.B. Eickhoff:** None. **T. Jiang:** None. **J. Sui:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.09/BB60

Topic: I.07. Data Analysis and Statistics

Support: NIH R21 MH096239
California Capital Equity LLC
UCLA Bioengineering Fellowship

Title: EEG source localization algorithm gFOTV and adaptive causal modeling in study of selective attention

Authors: *H. ZHOU[1], C. M. HABER[3], A. IRIMIA[4], G. V. SIMPSON[5], M. S. COHEN[2], A. LENARTOWICZ[2], W. LIU[1];
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Abstract: Compared to fMRI, EEG has higher temporal resolution that enables the analysis of the brain dynamics on a millisecond-scale. EEG source localization algorithms inherit the high temporal resolution of EEG and alleviate the volume conduction problem by reconstructing current densities on cortex. Our previously published method, graph fractional-order total variation (gFOTV), achieves a better performance than many other state-of-the-art methods such as MNE and sLORETA, in terms of less localization error and being able to preserve the spatial smoothness of source. Since brain signals are non-stationary, brain dynamics change with time, to establish the causality among cortical regions at each time point we further apply Kalman filter-based granger causality, which has no assumption about the stationarity of signals. In this study, we recorded 256-channel EEG from 34 subjects alternating between sustained visual and auditory selective attention, creating four conditions of interest: visual attending, visual ignoring, auditory attending and auditory ignoring. Within each condition we sought to identify underlying sources and connectivity, testing whether unique or common network components are involved in selective attention across modalities. We applied gFOTV to the event-related potential (ERP) of each condition to reconstruct source signals. In the source space, we identified regions of interest (RoI) based on anatomical priors of attention systems and group-level, omnibus t-tests. We then used adaptive granger causality to establish causal flow of information across RoIs, across the ERP in each condition. The great number of accessible EEG sensors and trials, an effective source localization algorithm, as well as adaptive causal modeling, all enabling an accurate connectivity analysis on a scale of milliseconds. The results show that the common attention sources for both modalities were located in the superior parietal lobe, and the junction of parietal and temporal lobes. In the auditory modality, attending was associated with sources in the temporal plane, inferior temporal and inferior parietal cortices, while ignoring was associated with sources in the junction of the superior parietal lobe and supramarginal lobe. In the visual domain, attending was associated with sources in the inferior parietal lobe, supramarginal gyrus and associative occipital cortex, whereas ignoring was associated with smaller source activities in occipital and frontal cortices. The results suggest that both unique and common information pathways exist for attending and ignoring across modalities.

Disclosures: H. Zhou: None. **C.M. Haber:** None. **A. Irimia:** None. **G.V. Simpson:** None. **M.S. Cohen:** None. **A. Lenartowicz:** None. **W. Liu:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.10/BB61

Topic: I.07. Data Analysis and Statistics

Support: 5T32MH067631-14

Title: Tensor-based brain network embedding in a transdiagnostic psychiatric cohort

Authors: *P. J. THOMAS[1], B. CAO[2], A. LEOW[1], P. S. YU[1], K. PHAN[1], O. A. AJILORE[1]; [1]Univ. of Illinois at Chicago, Chicago, IL; [2]Facebook, Inc, Menlo Park, CA

Abstract: The use of brain network data in the classification and understanding of brain disorders is an ongoing challenge in the field of neuroimaging research. Such networks are represented as a set of vertices (brain regions) and edges (connections between brain regions), which are defined based on imaging modality. A brain network may be represented by an adjacency matrix, $A \in R^{m \times m}$, where m is the number of brain regions, and each entry $A(i,j)$ is the edge weight connecting regions i and j. Because of the complex structure of network data, it cannot be directly analyzed with traditional classification approaches. Commonly, features that describe each graph are computed, and these graph theoretical measures are used for analysis. However, predefining features limits access to potentially informative latent network structure. Tensor-based brain network embedding (t-BNE) is a tensor factorization based classification method that addresses this disparity. In t-BNE, classifier training is allowed to interact with original brain network data, so latent network factors are accessible in the learning process. In addition, the derived vertex factors are accessible, and may be used to understand which network vertices are most influential in subject classification. Here, we use t-BNE on a structural diffusion tensor magnetic resonance imaging (dtMRI) dataset from a transdiagnostic cohort of healthy controls (HC, n=23) and patients (PT, n=66) with internalizing psychopathologies (e.g., depression and anxiety). t-BNE was able to classify subjects as PT or HC with an accuracy of 72.2% (training set n=71, testing set n=18). In addition, t-BNE identified vertex factors most influential in classification. Some of the most influential nodes include the left and right precuneus, and the left mid cingulate. These regions, as major constituents of the default mode network, have been previously implicated as part of abnormal networks in major depressive and anxiety disorders, common internalizing psychopathologies. These findings can not only inform the understanding of psychopathology, but may also lead to more accurate diagnosis and treatment selection for psychiatric illnesses.

Disclosures: **P.J. Thomas:** None. **A. Leow:** None. **P.S. Yu:** None. **K. Phan:** None. **O.A. Ajilore:** None. **B. Cao:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.11/BB62

Topic: I.07. Data Analysis and Statistics

Support: This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1324585.
National Institute on Drug Abuse (DA044121)

Title: Uni- versus multivariable identification of grey matter biomarkers in osteoarthritis

Authors: *A. D. VIGOTSKY[1], J. BARROSO[2], A. V. APKARIAN[3];
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[3]Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Pain is a debilitating and persistent symptom of osteoarthritis (OA). Previous work suggests that OA patients have large disruptions in grey matter that are distinct from other chronic pain conditions; the magnitudes of these disruptions are related to pain duration. To derive estimates of disruption, simple linear regression is commonly used; however, the multidimensional nature of the brain its substructures may not be fully captured by such approaches. Thus, we aim to identify brain structural biomarkers that distinguish hip and knee OA patients from pain-free controls using both univariable and multivariable approaches. Structural T1 magnetic resonance imaging (MRI) scans of 36 healthy controls (age = 60 ± 8 years), 24 hip OA patients (age = 59 ± 8 years), and 91 knee OA patients (age = 66 ± 7 years) were used for analyses. Knee patients were split into a training (n = 46) and validation group (n=45), which were matched on 14 covariates. We used the AAL atlas to obtain volumes of 90 regions of interest (ROI), and ANCOVA (univariable) and logistic regression (multivariable) to evaluate the ability of ROI volume to predict group membership and ROI differences between groups. The univariable analyses consisted of a single ROI, with age and sex as covariates of no interest. Because the number ROIs were greater than the number of participants, we used the Lasso for variable selection, for which lambda was chosen via 10-fold cross-validation.
The Lasso and ANCOVA produced several overlapping ROIs, but the Lasso also produced non-zero parameter estimates for structures that had negligible t-statistics in the ANCOVA. For example, in the knee training group, hippocampus had a relatively large weight in the Lasso but $t < 1$ in the ANCOVA. Importantly, the Lasso performed favorably in predicting out-of-sample group participation (AUROC ≈ 0.8), suggesting that the model was not overfit and that the selected parameters are important predictors.
Our results show that multivariable approaches to biomarker selection, such as the Lasso, may be more sensitive than univariable modeling. These findings suggest that the brains of patients with

OA likely undergo changes in multiple dimensions which cannot be fully captured with unidimensional approaches.

Disclosures: A.D. Vigotsky: None. **J. Barroso:** None. **A.V. Apkarian:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.12/BB63

Topic: I.07. Data Analysis and Statistics

Support: CIHR Program: Foundation Grant #148453
Brain Canada Program: Platform Support Grants
BrainsCAN Program: CFREF Imaging Core Research Grant

Title: Phase-based macrovascular filtering from gradient echo bold fMRI reduces orientation dependence

Authors: *O. W. STANLEY[1,2], A. B. KUURSTRA[2], R. S. MENON[1,2];
[1]Med. Biophysics, The Univ. of Western Ontario, London, ON, Canada; [2]Ctr. for Functional and Metabolic Mapping, Robarts Res. Inst., London, ON, Canada

Abstract: High-resolution blood oxygenation level dependent (BOLD) based MRI studies have, recently, become a powerful tool to non-invasively probe the sub-millimeter functional organization of human cortex. However, consensus within the MRI community regarding the ideal MRI acquisition remains divided. One method, gradient-echo echo planar imaging (GE-EPI) is sensitive to both the macro and microvasculature signal but, along the cortical surface, is dominated by signal from large draining vessels. This signal must be removed to allow for accurate laminar studies of the cortex using GE-EPI. One way of achieving this is through the use of the GE-EPI phase as a macrovascular filter [Menon, MRM. 2002] since phase data only contains BOLD signal in voxels dominated by large vessels.
This abstract examines the effect of this filtering in relation to orientation of the cortex with the main magnetic field, B_0. To investigate this, we collected functional GE-EPI data from seven subjects on a neuro-optimized 7T scanner at 800um while observing an 8Hz contrast reversing checkerboard. GE-EPI phase data was collected and used to create a macrovascular filtered time series through phase regression. Both the filtered and unfiltered time series were cleaned and fit to a general linear model. Anatomical imaging (0.75 mm isotropic MP2RAGE) was also collected and used for surface segmentation using Freesurfer. Additionally, a multi-echo gradient echo sequence was collected (0.31x0.31x0.8mm) to calculate R_2^* for identification of venous vasculature.
Figure 1 shows the % signal change for the filtered and unfiltered data. There is a reduction in

the pial vessel signal in the filtered case as the high activation near areas of high R_2^* are removed. This reduction overlaps with areas where the orientation of the cortex is close to parallel to B_0 and therefore pial veins will dominate the voxel signal. This work demonstrates that phase regression of GE-EPI time-series reduces the effect of orientation on task-based laminar studies by removing the influence of draining vessels when the cortex is close to parallel to B_0.

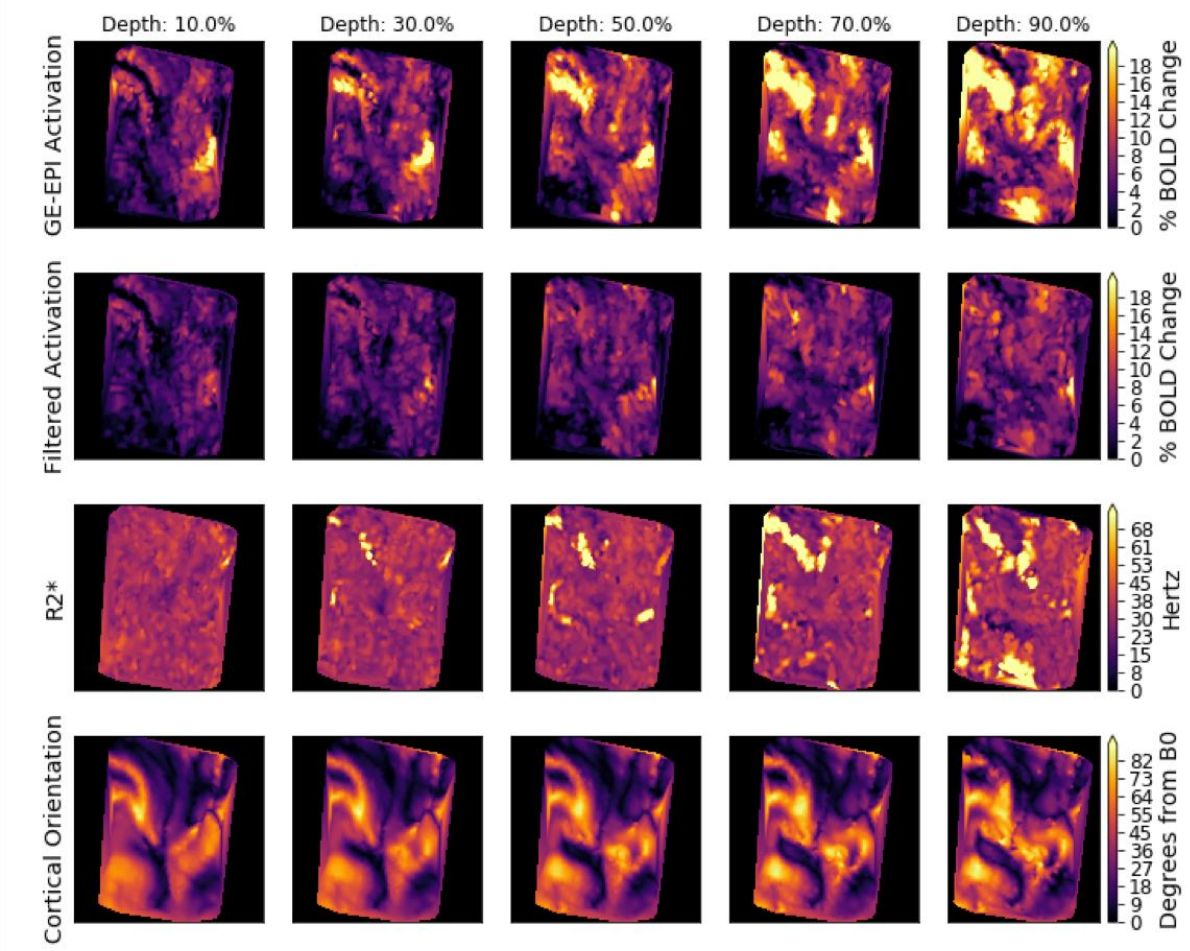

Figure 1 - Surface patch of an example subject at five equidistant depths. Top Row: GE-EPI % signal change. Second Row: GE-EPI % signal change after phase based macrovascular filtering. Third Row: R_2^* projected across the cortex as an anatomical vessel localizer. Fourth Row: Orientation of the surface relative to B_0.

Disclosures: **O.W. Stanley:** None. **A.B. Kuurstra:** None. **R.S. Menon:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.13/BB64

Topic: I.07. Data Analysis and Statistics

Support: P01 HD001994-46

Title: The Haskins pediatric atlas: A comparison of spatial normalizations among MRI templates

Authors: *P. J. MOLFESE[1], D. GLEN[2], R. W. COX[2], P. A. BANDETTINI[3]; [1]NIMH/NIH, Bethesda, MD; [2]NIMH / NIH, Bethesda, MD; [3]Section on Functional Imaging Methods, NIMH-NIH, Bethesda, MD

Abstract: Spatial normalization is fundamental to multi-subject fMRI experiments as it facilitates a common space in which group analyses are performed. Commonly, experimental data is aligned to either a group template (e.g. MNI-152) or to an individual template (e.g. “Colin” N27); however, problems have been identified with using these templates derived from adults with pediatric populations due to age related-variability in grey and white matter (Fonov et al., 2011; Muzik et al., 2000). Although some laboratories have attempted to address these problems by using study-specific templates (Wilke et al., 2008; Huang et al., 2010), these limit comparisons across studies and fail to provide cortical and subcortical segmentations. To address these limitations, we introduced a pediatric group template in 2015 and corresponding atlas derived from high-resolution anatomical scans of 74 children to aid in registration and group comparisons for pediatric populations. In this presentation, we compare our template/atlas combination against both adult and pediatric templates (MNI, MNI pediatric, Colin27) by “normalizing” 301 brains from the Healthy Brain Network / Child Mind Institute sample to each template and reporting different metrics of deformation distance to each template. Results show that normalization with the Haskins Pediatric Template results in smaller deformation distances and better registration of subcortical regions than all other templates tested.

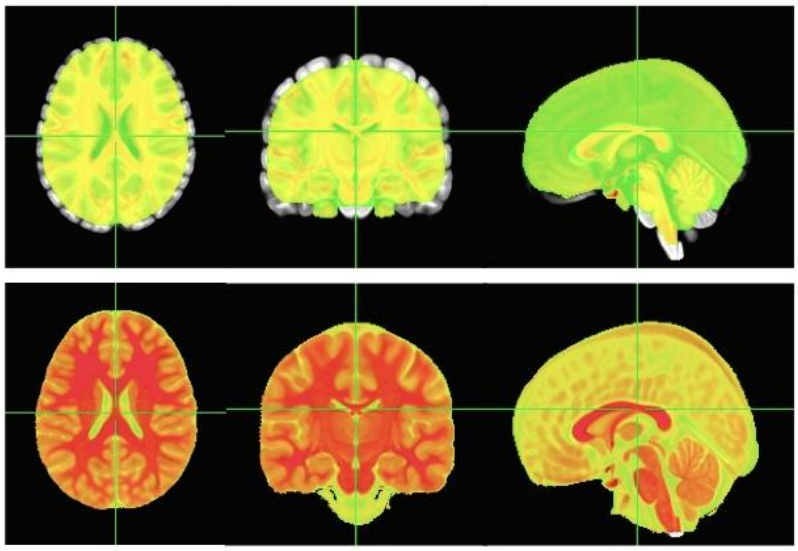

Figure 1 (Top): Shows Haskins template in green overlaid on MNI152 template, depicting the smaller size of Haskins.

Figure 1 (Bottom): Shows pediatric MNI in red overlaid on MNI152 template, showing the two are similarly sized

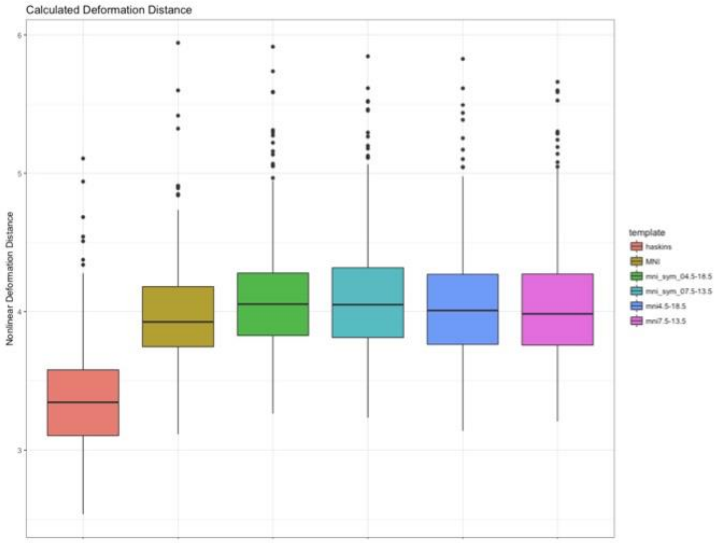

Figure 2: Deformation distances of CMI sample composed of 301 brains normalized to each of six templates. Result show that data registered to the Haskins template show smaller overall deformation distances than to any of the MNI or MNI pediatric templates.

Disclosures: P.J. Molfese: None. **D. Glen:** None. **R.W. Cox:** None. **P.A. Bandettini:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.14/BB65

Topic: I.07. Data Analysis and Statistics

Support: R01 MH071589
R01 MH112517

Title: Geodesic distances between functional connectivity matrices: A geometry-aware approach

Authors: *M. VENKATESH[1], J. JAJA[1], L. PESSOA[2];
[1]Electrical and Computer Engin., Univ. of Maryland, Col. Park, College Park, MD; [2]Psychology, Univ. of Maryland at Col. Park Dept. of Psychology, College Park, MD

Abstract: Functional connectivity (FC), which can be estimated via correlating time series data between pairs of brain regions, indicates that the brain can be segregated into functional subnetworks. Matrices capturing the FC between all brain region pairs have been used as a form of "fingerprint" to accurately determine participant identity. To do so, an unknown participant's FC matrix is labeled based on the "closest" participant's FC in the database. However, in these and related analyses, the geometry of FC matrices has not been adequately handled. By construction, sample covariance/correlation matrices lie on the positive semidefinite cone: their geometry is non-Euclidean. Yet, similarity between FC matrices has been measured typically using Pearson correlation (that is, the correlation coefficient of the correlation matrices), or via Euclidean distance. Although these measures serve as proxies for the true distances between FC matrices, they are not entirely appropriate for the manifold on which FC matrices lie. Here, we investigated participant identification by employing geometry-aware methods. The geodesic distance, an invariant metric for the space of covariance matrices, was computed based on the eigenvalues of the FC matrices. We tested our approach in a sample of N=100 participants of the Human Connectome Project dataset, and investigated resting-state and seven task conditions acquired with functional MRI. Using the geodesic distance, identification accuracy was over 95% on resting-state data, and exceeded Pearson correlation by 20%. For whole-brain FCs, accuracy improved on the remaining tasks by between 2% and as much as 20%. We also investigated identification using pairs of subnetworks (say, dorsal attention plus visual), and particular combinations improved over whole-brain participant identification by over 10%. The geodesic distance also outperformed Pearson correlation when the former employed a fourth of the data as the latter. Finally, low-dimensional distance visualizations based on the geodesic approach uncovered the geometry of task FCs in relation to resting-state FCs, thus providing an understanding on how the FC geometry affected identification. In conclusion, the geodesic distance not only improves identification accuracy but also provides insights into task and

resting-state FC geometry. A deeper understanding of such geometry can benefit further analyses of functional connectivity by, for example, helping determine the type of clustering technique used to identify similar brain states or the non-linearity of a classifier used to predict participant groups.

Disclosures: M. Venkatesh: None. **J. Jaja:** None. **L. Pessoa:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.15/BB66

Topic: I.07. Data Analysis and Statistics

Support: Netherlands Organization for Scientific Research 016.Veni.178.048

Title: The beating brain: A rapid MRI-based technique to characterize whole brain cardiac pulsations

Authors: *D. HERMES[1], A. KERR[2], H. WU[3], B. A. WANDELL[4];
[1]Mayo Clin., Rochester, MN; [2]Dept. of Electrical Engin., [4]Psychology, [3]Stanford Univ., Stanford, CA

Abstract: Cerebrospinal fluid (CSF) and blood flow through the brain, driven by the cardiac pulse cycle. Several noninvasive magnetic resonance imaging (MRI) methods can measure typical and atypical fluid dynamics, but techniques need further improvement to rapidly and simultaneously characterize the whole-brain distribution of cardiac pulsations, including CSF spaces, surface vessels and parenchymal vessels. We build upon recent developments in MR physics and use a fast T2* weighted simultaneous multislice (SMS) technique to assess blood and CSF flow dynamics.

Six subjects were scanned with a 3.5 minute T_2^* weighted SMS sequence at 3T MRI. In this sequence, every voxel was sampled within multiple 50 ms windows, which were aligned to the cardiac pulse measured simultaneously by a photoplethysmogram (PPG).

Highly reliably whole-brain cardiac pulsations were observed in CSF and blood across scans and subjects (test-retest reliability within subjects $R^2>0.80$). A model-based approach allowed us to distinguish cardiac pulsations in CSF and blood and identify the temporal dynamics. Early cardiac pulsations were observed in the carotid and basilar arteries and in branches of the anterior, posterior and middle cerebral arteries. Later cardiac pulsations were observed in the superior sagittal, transverse and straight sinuses. CSF pulsations were observed in the ventricles and sub-arachnoid spaces.

These data are a proof of principle that we can measure brain-wide cardiac pulsations in the CSF and blood, rapidly and noninvasively at 3T MRI. These measurements allowed an estimate of the

phase of the cardiac pulsations in the CSF relative to those in the arteries, which is thought to be an estimate of the local intracranial impedance. This technique can provide new insights or diagnostics for diseases where fluid dynamics are otherwise difficult to measure.

Disclosures: D. Hermes: None. **A. Kerr:** None. **H. Wu:** None. **B.A. Wandell:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.16/BB67

Topic: I.07. Data Analysis and Statistics

Support: NIH GM130461
NSF/EPSCoR 1632849

Title: Effects of visual noise on the performance and robustness of multivariate classifiers in EEG

Authors: *P. LIM, K. KUNTZELMAN, L. N. BANDEL, M. BEHRENDT, M. R. JOHNSON; Ctr. for Brain, Biol. and Behavior, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: In this study, we investigated how differing levels of visual noise would affect the performance of a multivariate pattern analysis (MVPA) classifier, and specifically whether there would be a benefit to training a classifier on noisier trials versus less noisy ones. Using a sample EEG dataset, we trained classifiers to decode the category of items being presented (faces or scenes), with different levels of noise present (zero noise, medium noise, high noise). First, one classifier was trained for each level of noise (i.e., one classifier was trained to decode item category on all zero-noise trials, a second classifier was trained to decode item category on medium-noise trials, and so on). Then, these pre-trained classifiers were applied to trials from all noise levels (i.e., the classifier initially trained on zero-noise trials was used to decode medium- and high-noise trials, and so on for each of the three classifiers). As expected, during the initial training, decoding accuracy was highest for trials with the lowest noise levels. Also as expected, each classifier performed best on trials with the same noise level the classifier was initially trained on. In general, classifiers also performed better on conditions with similar levels of noise; i.e., the zero-noise-trained classifier performed better on medium-noise trials than high-noise trials, and the high-noise-trained classifier performed better on medium-noise trials than zero-noise trials. However, and perhaps more notably, there was a general advantage for classifiers trained on higher levels of noise when classifying data from an adjacent noise-level condition; in other words, the medium-noise-trained classifier performed better on zero-noise trials than the zero-noise-trained classifier performed on medium-noise trials, and similarly for the other noise levels. This suggests that in general, if one intends to develop classifiers that are robust to

different levels of visual noise, the advantages of training the classifier to expect a certain level of noise outweigh the potential advantages of cleaner training examples in a lower-noise training dataset.

Disclosures: P. Lim: None. **M.R. Johnson:** None. **K. Kuntzelman:** None. **L.N. Bandel:** None. **M. Behrendt:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.17/BB68

Topic: I.07. Data Analysis and Statistics

Title: Are you local? In most neuroimaging studies, computer says no

Authors: *T. JOHNSTONE;
Swinburne Univ. of Technol., Melbourne, Australia

Abstract: Researchers have rightly highlighted the importance of anatomical precision in functional MRI (e.g. Devlin & Poldrack, 2007), yet if the aim is localizing function, then researchers need to actually *test* functional localization. Currently this is almost never done, imnplying that much of what we think we know about functional localization from fMRI might be wrong.
The current default method for "localizing" fMRI activation is unidirectional voxelwise thresholding of a statistical map. This approach implicitly accepts the null hypothesis that areas falling below threshold are not activated by the condition of interest. Accepting the null based on a unidirectional statistical threshold would not be accepted in other fields of science but remains the basis for localization claims in the vast majority of fMRI studies.
The problem is magnified by most fMRI studies being under-powered (Thirion et al., 2007), so that false negatives outnumber true positives. This has an alarming implication: The degree of apparent localization is greater (i.e. thresholded brain activation more focal) the more underpowered a study is! Given much higher statistical power, in studies with larger N for example, much more extensive, less focal regions of activation might be found. This is exactly what has been reported (Gonzalez-Castillo et al., 2012; Thyreau et al., 2012). Finding the same brain region activated over multiple experiments (e.g. in meta-analyses) offers little protection from false inferences of functional localization because the same regions of the brain might exceed threshold simply because they are consistently low noise regions (i.e. focal SNR hotspots).
Here I illustrate, with publicly shared fMRI data, explicit tests that can be used to generate Functional Localisation Maps (FLMs). In the first method, percent signal change values are extracted from suprathreshold clusters from a standard voxelwise analysis. Voxelwise

permutation-based equivalence tests are then performed to identify those voxels elsewhere in the brain that show significantly less activation. A second method uses spatial mixture models that assign an explicit estimated probability that any one voxel belongs to the distributions of activated or non-activated voxels (Woolrich, Behrens, Beckmann, & Smith, 2005). The third method estimates voxelwise Bayes Factors, which provide evidence for both activation and no-activation for every voxel. The relative strengths and weaknesses of each approach are discussed. Although imperfect, all offer better protection against false claims of functional localization than the traditional unidirectional thresholding approach.

Disclosures: T. Johnstone: None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.18/BB69

Topic: I.07. Data Analysis and Statistics

Title: Tract edge diffusion statistics; measuring diffusivity at the boundary of the white matter

Authors: *A. M. AZOR[1], D. J. SHARP[1], P. J. HELLYER[2];
[1]Imperial Col. London, London, United Kingdom; [2]King's Col. London, London, United Kingdom

Abstract: IntroductionRecent advances in the field of magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) in particular, have enabled detection of pathology-specific features such as microstructural changes in the white matter's (WM) axons and myelination. DTI is commonly used in clinical investigations of traumatic brain injuries (TBI), epilepsy, neurodegenerative disorders and stroke1. In clinical studies, researches are typically interested in group comparisons, and standardized automated analysis. Therefore, individual images are normalized and warped to a standard space, and WM diffusivity metrics are measured away from mixed-intensity signals and at the centre of the WM tracts to ensure pure WM signals2. However, many of the clinical cases lead to blurring and degeneration at the edge of WM tracts, either closer to cerebral spinal fluid-filled cavities3 or at the white-grey matter boundary4. In these cases, there is no validated and standardized automated way to measure microstructural damage at the WM interface.
MethodsHere, we present the validation of a new fully automated method to measure diffusivity at the edge of specific WM tracts, or similarly, diffusivity in subject-specific space with no group transformation involved. Tract Edge Diffusion Statistics (TEDS) is constrained by underlying anatomy and performs DTI analysis in native B0 space. Boundary-based registration is used to register a native T1 image to the corresponding native diffusion image. Non-linear registration couple with the boundary-based registration matrix yields a warp field to move standard template

tracts into native space. Having both the T1 and the template image in diffusion can help guide the segmentation, by restricting it to the underlying anatomy.
Results and ConclusionThe method is validated in controls with normal looking brains and in cases of TBI patients where tracts are large enough to delimit a 2-side boundary. TEDS performs best in specific larger tracts where boundary resolution is not lost once it is warped to B0. Moving forwards, this method will be further optimized to be applicable across all WM tracts and applied to a large cohort of TBI patients to understand how diffusivity at the edge of tracts 1) compares to other methods such as tract-based spatial statistics and 2) is affected by pathology and trauma.

Disclosures: **A.M. Azor:** None. **D.J. Sharp:** None. **P.J. Hellyer:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.19/BB70

Topic: I.07. Data Analysis and Statistics

Support: CCDC Army Research Laboratory contract W911NF-17-2-0158.

Title: Avalanches in human brain dynamics dissociate task specific changes

Authors: K. BANSAL[1,2], J. O. GARCIA[2], N. LAUHARATANAHIRUN[2], S. E. MULDOON[4], P. SAJDA[1], *J. M. VETTEL[3];
[1]Biomed. Engin., Columbia Univ., New York, NY; [3]Future Soldier Technologies Div., [2]CCDC Army Res. Lab., Aberdeen Proving Ground, MD; [4]Univ. at Buffalo, SUNY, Buffalo, NY

Abstract: Avalanches in a neuronal system are large amplitude bursts that emerge due to cascading spatiotemporal activity. Neuronal avalanches have been studied in a variety of systems ranging from small assemblies in neuronal cultures to resting state cortical dynamics in human neuroimaging, and results have revealed the macroscopic organization of brain dynamics. In resting state neuroimaging data, avalanches have previously shown to have a scale-free organization, which is believed to be crucial for dynamical and functional diversity of the brain. A few recent studies have shown the emergence of scale-free brain organization during functional task processing, but its relationship to human behavior remains unknown. In this work, we analyzed avalanches in the EEG activity of healthy humans during resting state and two distinct task states that involved viewing emotionally charged images. One task required participants to passively view images, while the second required an active judgment about the image. First, our results replicated the previous resting state findings, identifying scale-free organization where avalanche organization follows a power-law distribution. Second, our results revealed that the global dynamics during task processing also demonstrate scale-free

organization; however, the local features of this distribution are not universal and vary with complexity of task (passive viewing versus emotional judgment). Critically, this observed variability in the localized avalanches accounts for individual differences in task performance, suggesting that avalanches may capture the diversity in behavior. We propose that avalanches provide a robust tool to assess variability in cognitive states and present a first link between avalanche dynamics and human behavior.

Disclosures: **K. Bansal:** None. **J.O. Garcia:** None. **N. Lauharatanahirun:** None. **S.E. Muldoon:** None. **P. Sajda:** None. **J.M. Vettel:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.20/BB71

Topic: I.07. Data Analysis and Statistics

Support: Canada First Research Excellence Fund (BrainsCAN to Western University)
Canadian Institutes of Health Research (PJT 159520 to J.D.)
James S. McDonnell Foundation (Scholar award to J.D.)

Title: Evaluation of functional brain parcellation methods using a multi-domain task battery

Authors: *D. ZHI[1], M. KING[3], C. R. HERNANDEZ-CASTILLO[1], R. IVRY[4], J. DIEDRICHSEN[2];
[2]Brain and Mind Inst., [1]Western Univ., London, ON, Canada; [3]Univ. of California, Berkeley, CA; [4]Univ. California, Berkeley, CA

Abstract: Human brain parcellation aims to identify distinct functional regions to facilitate the understanding of the human brain as a modular structure. Practically, such regions are important to guide the analysis of human functional magnetic resonance imaging (fMRI) data. Numerous parcellations for the human neocortex and cerebellum have been proposed over the last years, based on anatomical boundaries, functional task-based data, or functional resting-state connectivity. However, an objective evaluation of different parcellations and methods is currently missing. In this paper, we explore the pros and cons of different parcellation methods applied for finding functional boundaries in human brain. Our evaluation method relies on a novel Multi-Domain Task Battery (MDTB) data set [King et al., 2018, BioRxiv], which contains a wide range of tasks involving multiple motor, social, and cognitive functions. During scanning sessions, each of the 24 participants (16 females, 8 males, age mean=23.8) was scanned in four 90-minutes sessions to perform task set A in the first two sessions and B in the last two sessions (17 tasks for each, 8 tasks in common). Ultimately, each voxel could be characterized by an activity profile across the 47 unique task conditions. A good brain parcellation should separate

regions that have distinct functional profiles, while keeping regions with correlated profiles together. To account for the fact that function varies in a smooth fashion across the cortical sheet, we calculated the distance-controlled boundary coefficient (DCBC), which compares the correlation between any two locations with the same distance on the cortical surface. A higher functional correlation for within-region pairs as compared to between-regions pairs indicates that the brain parcellation was able to accurately identify boundaries in functional specialization. Given this evaluation criterion, we compared different brain parcellation methods for cerebellar and cortical data. First, we found that parcellations based on task-based data can outperform those using resting-state data to predict functional boundaries. We then compared various clustering algorithms, including convex semi-nonnegative matrix factorization and hierarchical clustering methods, for the task-based data. Our results show that spectral clustering using cosine distance-based affinity matrix provides improvements compared to the others. We also provide the detailed analysis from a topological perspective to explain why cosine distance is better for functional parcellation. Finally, we compare methods of deriving brain parcellations on individual and group averaged data.

Disclosures: D. Zhi: None. **M. King:** None. **C.R. Hernandez-Castillo:** None. **R. Ivry:** None. **J. Diedrichsen:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.21/BB72

Topic: I.07. Data Analysis and Statistics

Title: Advancing legacy fMRI analyses: Towards fieldmap-free susceptibility distortion correction of GRE EPI data

Authors: *C. FONTENEAU[1,2], L. J. JI[1,2,3], A. HOWELL[1,2,3], G. REPOVS[4], E. W. DICKIE[5], T. S. COALSON[6], J. ANDERSSON[7], M. F. GLASSER[6,8], A. ANTICEVIC[1,2,3,9];
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Abstract: Gradient Recalled Echo (GRE) functional MRI (fMRI) is the dominant method for mapping human brain activity non-invasively. However, to maximize the impact of fMRI

research, it is vital for neuroimaging analyses to respect the sheet-like 2D cortical and 3D globular subcortical geometries of the human brain when making comparisons across subjects or smoothing data. These surface and CIFTI-based approaches, rely on precise alignment between undistorted anatomical (e.g. T1-weighted, T1w) and distorted Echo-Planar Imaging (EPI) fMRI data. Removing this distortion in the phase encoding (PE) direction induced by inhomogeneities in the b0 field enables accurate mapping of cerebral cortical signal onto surface models. Multiple methods have been developed to estimate the field inhomogeneity map, such as PE polarity techniques (acquiring spin echo images with opposite PE directions) and field mapping techniques (measuring the phase evolution in time between two close gradient echo acquisitions). However, many past and even some current human EPI protocols omit the necessary acquisitions to apply generally accepted susceptibility distortion correction (SDC) methods (i.e. legacy data). Recently, experimental nonlinear EPI deformation approaches have emerged, attempting to circumvent the need for extra acquisitions for fieldmap estimation by using the T1w image as the undistorted target. Our goal was to develop a robust fieldmap-free method for legacy data, which improves the alignment of EPI data with T1w image similarly to generally accepted methods, but without inducing other spurious distortions. Our approach is to first precisely rigidly align the EPI image to the T1w image using boundary-based registration (BBR); then brain-mask both EPI (losely) and T1w (tightly) images, invert the contrast of the T1w image and match its histogram to the EPI, and compute a map of gradient echo EPI signal loss; and finally iteratively nonlinearly register the EPI to the T1w image. We are evaluating this approach with HCP-style data collected on a TimTrio 3T Siemens at Yale. Incisive evaluation requires both inspection of the alignment between EPI and T1w images and comparison between non-fieldmap corrected results and generally accepted fieldmap corrected results, ideally using reversed PE directions to look for subtle deviations between the respective distortion corrections. Additionally, the fMRI neural signal itself should follow the T1w-defined cortical ribbon as assessed by signal spatial ICA components. We expect a robust fieldmap-free correction method to open a large new cache of data to modern surface and CIFTI-based brain imaging analysis methods.

Disclosures: **C. Fonteneau:** None. **L.J. Ji:** None. **A. Howell:** None. **G. Repovs:** F. Consulting Fees (e.g., advisory boards); BlackThorn Therapeutics. **E.W. Dickie:** None. **T.S. Coalson:** None. **J. Andersson:** None. **M.F. Glasser:** None. **A. Anticevic:** 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.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.22/BB73

Topic: I.07. Data Analysis and Statistics

Title: Using global t-SNE to reveal global structure of human brain atlas

Authors: *Y. ZHOU[1], T. O. SHARPEE[2];
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Abstract: The t-distributed Stochastic Neighbor Embedding (t-SNE) method is one of the leading techniques for data visualization and clustering. This method finds lower dimensional embeddings of data points while minimizing distortions in distances between neighboring data points. By construction, t-SNE discards information about large scale structure of the data. We show that adding a global cost function to the t-SNE cost function makes it possible to cluster the data while preserving global inter-cluster data structure. We test the new "global t-SNE" (g-SNE) method on one synthetic and two real data sets on flowers and human brain cells which have significant and meaningful global structures. In all cases, g-SNE outperforms t-SNE in preserving the global structure, and g-SNE outperforms UMAP in preserving the inter-cluster structure of brain atlas. The weight parameter λ of the global cost function determines the balance between local and global distances preservations. For the human brain atlas data set, we the find optimal λ is different in different brain donors, which means the λ might serve as an indicator of brain states.

Disclosures: Y. Zhou: None. **T.O. Sharpee:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.23/BB74

Topic: I.07. Data Analysis and Statistics

Title: A predictive approach to personality: Using machine learning to build better models

Authors: *J. E. KOBSA, A. TAVAKKOLI;
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Abstract: Machine learning techniques are growing more common in neuroinformatics and psychoinformatics. The benefit of machine learning techniques in data analysis is that statistical models are valued for their ability to predict out-of-sample, unseen data, rather than for their fit to the observed data. This study aimed to evaluate the machine learning approach to data analysis by applying it to a novel data set in which outcome measures were used to predict factors of the Five Factor Model (FFM) of personality. The FFM includes the five traits that underly human personality. Abundant previous literature has correlated these traits with various demographic and social outcome measures. However, awareness has grown recently that correlations are

susceptible to overfitting and collinearity of variables, which may seem to support false relationships. This study specifically aimed (1) test the replicability of previous findings on the associations between personality traits of the Five Factor Model and various outcomes on a new dataset and (2) test an approach focused on prediction rather than on explanation using machine learning techniques. This study collected a new dataset (n = 134) consisting of measures of personality traits and measures of various outcomes and developed predictive models for each personality trait. For each trait, one model was developed using traditional multiple linear regression, and another regression model was developed using variables selected by LASSO cross-validation, a machine learning technique. The models were then tested on out-of-sample data and the error of their predictions compared. For four of the five personality traits, the LASSO-based model predicted out-of-sample data with less error than the traditional model, suggesting that machine learning techniques can be used to build better predictive models.

Disclosures: **J.E. Kobsa:** None. **A. Tavakkoli:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.24/BB75

Topic: I.07. Data Analysis and Statistics

Support: JSPS KAKENHI Grant Numbers JP18K04184
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Title: Microsaccade detection method using a non-Gaussian state-space model

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Abstract: It is well known that our eyes are constantly moving in very small motions even when gazing at a point in the field of view. Such involuntary small eye movement is called fixational eye movements and is mainly composed of three kinds of components: microsaccades, ocular drift, and tremor. It is very important to know the characteristics of fixational eye movements because they are closely related to human cognitive function. To detect a microsaccade, a low-pass differential filter is generally used, but the detection accuracy can lower depending on the threshold setting. Recently, we have proposed a method for detecting the microsaccade period by statistical testing the average value of certain feature quantities obtained from order statistics when the time scale is changed. In this method, it is not necessary to set the threshold by trial and error; however, a precise trace of the microsaccade is still a challenge. In this report, we model

fixational eye movement using a state-space model and propose a new alternative method to detect a microsaccade and ocular drift and tremor simultaneously. We constructed the following state-space model of fixational eye movements: $x(n) = x(n-1) + v(n-1)$, $y(n) = x(n) + w(n)$, $v(n) \sim \text{Cauchy}(0,\sigma_x)$, and $w(n) \sim \text{Normal}(0,\sigma_y)$, where $x(n)$ is a drift component, whose mean value is estimated using a first-order trend model. Here, we assume that $v(n)$ follows the Cauchy distribution because the mean value suddenly changes on occasions as a result of the saccade component. $y(n)$ is the observation signal of fixational eye movement. Here, we assume that $w(n)$ has a normal distribution. To evaluate our proposed method, we used the eye movement signal measured when a light-gray cross-type fixation target was presented at the center of the screen with a gray background of medium luminance for 7 s. The results demonstrated that our proposed method could be used to estimate the jumping microsaccade components and accurately trace the slowly fluctuating drift component. In addition, overshoots and undershoots of microsaccades could be traced precisely. The characteristics of overshoot and undershoot of the saccade represent the characteristics of the eye movement control system and can aid in more accurate eye movement control analysis that goes beyond simply detecting the occurrence of the saccade. Our method also could estimate Bayesian prediction intervals of arbitrary value.

Disclosures: **H. Yoshida:** None. **K. Nagano:** None. **T. Kohama:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.25/BB76

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant MH116156

Title: Comparison between novel and established machine learning approaches to identify biotypes of posttraumatic stress

Authors: *J. L. NIELSON[1,2], T. KIRSH[2], B. E. COHEN[4], A. R. FERGUSON[5], T. C. NEYLAN[4], E. KUMMERFELD[2], S. MA[2,3];
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Abstract: Recent efforts to understand mental health disorders, including posttraumatic stress (PTS), have started to leverage advanced computational approaches for analyzing the mass of data that's been collected. The emerging field of computational psychiatry has begun to tackle the development of innovative approaches to understand PTS. This study aimed to identify

candidate biotypes of PTS that can be derived from a combination of machine learning approaches applied to clinical data from VA patients. ***Methods:*** Data were mined from the Mind Your Heart Study (MYH), collecting cardiovascular and neuropsychiatric data over 8 years (N=747) from VA patients with current (N=257) or no/past PTSD (N=477). Machine learning methods included latent class growth analysis (LCGA), Greedy Fast Causal Inference (GCFI), and non-linear principal component analysis (NL-PCA). Each method was performed independently by different team members on the same set of variables of PTSD symptoms, depression, social functioning, quality of life, physical activity and alcohol use. Cases without complete data across all years were dropped for GCFI and NL-PCA, giving sample sizes of 240 and 410, respectively. ***Results*****:** *LCGA:* The number of classes varied between three and five, identifying separate classes of patients that either showed improvements in symptoms, worsening of symptoms, or no change over time across the selected measures. *GCFI*: Alcohol use was independent of all other outcomes, whereas physical activity, depression and social functioning do not seem to drive their own values across time. However, PTSD symptoms appear to causally influence future PTSD symptoms as well as depression and social functioning, whereas quality of life and health appear to cause each other. *NL-PCA* found three orthogonal dimensions (PCs) that were conserved over time, accounting for 70% of the variance. PC1 is the relationship between health, PTSD symptoms, quality of life and depression that are inversely correlated to social functioning and physical activity. PC2 is a different relationship between PTSD symptoms and physical activity and depression, after the variance of PC1 was accounted for. PC3 was primarily alcohol use. ***Conclusions*****:** The biotypes we've detected represent conserved patterns within the MYH study, providing a candidate set of features that may be important to the stratification of PTS symptomatology. While these sets of biotypes may be true for the MYH study, they should be externally cross validated on separate datasets, such as those housed in the NIMH data archive (NDA), to determine their stability and reproducibility across different PTSD studies. **Funding**: NIMH R01 MH116156 (JLN).

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Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.26/BB77

Topic: I.07. Data Analysis and Statistics

Support: The European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement no. 616268 F-TRACT

The European Union's Horizon 2020 Framework Programme for Research and Innovation under Specific Grant Agreement No. 785907 (Human Brain Project SGA2)

Title: Functional and structural connectivity of the human brain: Synergy of results from direct electrical stimulation and diffusion magnetic resonance imaging

Authors: *M. JEDYNAK[1], L. TREBAUL[1], J.-D. LEMARECHAL[2], N. LABRA[3], P. DEMAN[1], V. TUYISENGE[1], F. TADEL[1], B. CHANTELOUP-FORÊT[1], C. SAUBAT[1], G. REYES MEJIA[1], C. POUPON[3], J.-F. MANGIN[3], O. DAVID[1], F. TRACT CONSORTIUM[1];
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Abstract: Functional tractography by means of Direct Electrical Stimulation (DES) provides information about functional connections between remote brain areas. Here, we perform group analysis of DES data and corroborate results obtained by multi-subject clustering of diffusion MRI High Angular Resolution Diffusion Imaging (dMRI HARDI)-derived fibers. The structural and functional data sets are complementary: fiber geometry that can be determined in the structural study cannot be found in the functional approach, which, in turn, provides information about the direction of signal transmission and propagation latency. We combine these two data sets to tests their mutual consistency and to find results inaccessible from within either method alone, namely signal propagation velocity.
First, we analyzed the HARDI data in order to derive the organization of fibers into bundles repetitive between individuals. We used data of 77 healthy human subjects from HARDI database called CONNECT/Archi that is distributed by the Human Brain Project. Second, in order to determine functional connectivity between remote brain areas we applied the procedure developed in the F-Tract project, i.e. we analyzed signals recorded with intracerebral (stereo-electroencephalographic) electrodes from nearly 300 drug-resistant epilepsy patients. Electrical stimulation in certain brain areas and simultaneous recordings in other areas allowed us to assess the existence of functional connectivity between these areas and characteristics (such as latency) of cortico-cortical potentials evoked by DES. Finally, we superimposed the obtained structural and functional data sets and tested how the geometry of the HARDI-derived fibers correlates with the spacial distribution of functional connectivity. We estimated the probability of signal transmission and its propagation latency along each fiber in both directions. High values of this probability can be interpreted as a confirmation of a given fiber existence, whereas low values might indicate that the fiber is a false-positive. Signal propagation velocity was obtained as a ratio of distance (length of a fiber) and propagation time (latency from the DES procedure). We relate the heterogeneous distribution of this velocity to nonuniform axonal myelination.
In summary, we present an integration of structural and functional data obtained from two distinct but complementary brain mapping methods, namely diffusion MRI and Direct Electrical Stimulation. This integrative approach allowed us to examine consistency between the two

methods and to find velocity of the signal propagation that could not be measured in either of these methods alone.

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Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.27/BB78

Topic: I.07. Data Analysis and Statistics

Title: The limit of mind reading: Challenging the added value of population multi-voxel pattern analysis (p-MVPA) to identify perceptual states

Authors: ***R. JABAKHANJI**[1], M. N. BALIKI[2], G. IANNETTI[3], A. V. APKARIAN[1]; [1]Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Rehabil. Inst. of Chicago, Chicago, IL; [3]Univ. Col. London, London, United Kingdom

Abstract: Across subjects population based Multi-Voxel Pattern Analysis (p-MVPA) of BOLD fMRI data claims to identify perceptual states for any individual given the specific voxel pattern, e.g. the “neurological Pain Signature” (NPS) is used to detect subjects in acute pain, and has been validated across experiments and labs. However, the fine-grained structure obtained by p-MVPA has not been contrasted with the common standard voxel-wise general linear modeling (GLM) outcomes. Here we test whether p-MVPA results are superior to GLM to identify acute pain, relative to other sensory states.We performed the dot product of the Neurological Pain Signature (NPS) [Wager et al 2013], the Pain-Preferring Voxels (PPV) [Liang et al 2019], as well as for the map generated by the meta-analysis tool Neurosynth for the term “Pain” with three different datasets. DS1: fMRI activation patterns of 14 participant subjected to 4 stimulus modalities: Painful heat, Touch, Audition, and Vision [Liang et al 2019]. DS2: fMRI activation patterns of 51 participant subjected to 2 stimulus modalities matched by reported-intensity: Painful heat, and Touch [Liang et al 2019]. DS3: fMRI activation patterns of 15 participant subjected to 2 stimulus modalities: Painful Heat, and Visual Rating [Baliki et al, 2009]. For each dataset-MVPA combination, we trained a classifier to identify painful stimuli. We then applied a series of spatial Gaussian filters (standard deviation ranging from 1 mm to 20 mm) to the original MVPA templates, effectively averaging out the fine-grained structure, and repeated the analysis. The accuracy of the original unfiltered classification varied between the different datasets and p-MVPA combinations, ranging from 0.63 (95%: 0.52-0.74) for DS2 dot PPV (painful heat vs touch), to 0.94 (95%: 0.71-1.00) for DS1 dot NPS (painful heat vs visual). In the spatially

filtered p-MVPA results, if local activation patterns hold state specific information, accuracy is expected to be reduced with increasing gaussian kernel width. However, we observed no reduction of accuracy with increased spatial filtering, implying that local activation patterns do not improve the discrimination or identification of perceptual states from that based on maps generated by linear voxel-wise analysis of fMRI responses. We thus demonstrate the limits of "mind reading" using MVPA of fMRI data across subjects.

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Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.28/BB79

Topic: I.06. Computation/ Modeling/ and Simulation

Title: Tools for integrative network neuroscience modeling of brain structure and activity

Authors: A. NANDA, ***M. RUBINOV**;
Vanderbilt Univ., Nashville, TN

Abstract: Network neuroscience is a young field that seeks to describe fundamental principles of brain-network architecture and function by analyzing brain-connectivity datasets with network-science models. Progress in this field depends on systematic specification and selection of models constrained by existing knowledge of brain-network organization. Model selection is in turn underpinned by uniform or unbiased sampling of networks with specified empirical constraints. However network sampling is a hard computational problem that, in general, has no known polynomial-time solution.
Here we developed a set of tools based on modern algorithms that make use of fast computers and analytical approximations to allow sampling of networks with a wide range of constraints. Our sampling methods fall into two broad classes. The first is based on constrained randomization of empirical data, and allows the direct sampling of networks with arbitrary accuracy. The second class is based on exact maximum-likelihood estimation, and satisfies network constraints in the ensemble average.
We applied these tools to analyze and model structural and functional connectivity datasets of several model species. We first illustrated how network features of interest, such the degree, gradients and modules (for structural connectivity), and activation and correlation patterns (for functional activity and connectivity) can be modeled as connectivity or activity densities. We demonstrated how constraints on some of these densities allow us to explain prominent properties of structural and functional brain connectivity, including the degree, time-varying connectivity and within-module (including default-mode network) densities.

Our toolbox fills an important gap in the presently standard analysis practice in network neuroscience. It enables investigators to constrain a wide range of empirical constraints, and thus allows discovery of new models that that are simpler, more plausible, or better fit the data. We encourage network neuroscientistis to adopt these tools in order to make such empirically grounded and statistically principled progress in this young field.

Disclosures: M. Rubinov: None. **A. Nanda:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.29/BB80

Topic: I.06. Computation/ Modeling/ and Simulation

Title: A numerical model of white matter tissue with progressive levels of demyelinization and axonal loss for the simulation of diffusion weighted images and the comparison of clinical imaging protocols

Authors: *C. DEL GRATTA, S. OLIVIERO;
Dept. of Neuroscience, Imaging and Clin. Sci., Gabriele D'Annunzio Univ., Chieti, Italy

Abstract: The purpose of this study is to quantify the sensitivity and the specificity of ordinary clinical DWI techniques to demyelination and axonal loss to identify a possible biomarker for demyelinating diseases.
A novel synthetic model of damaged WM has been developed, using three permeable compartments (intra-axonal, intra-myelin and extra-cellular space), each of them being characterized by its own wall permeability, diffusivity, and T2 relaxation. The ability to reveal the presence of axonal loss and demyelination and to distinguish one from the other, has been tested by evaluating the changes in the metrics of three diffusion models (i.e. DTI, DKI, NODDI) with different degrees of damage induced in the synthetic tissue. The results have been then quantified in terms of sensitivity and specificity indices. The impact of the acquisition protocol on these performances has been also evaluated.
All models are sensitive to both demyelination and axonal loss but sensitivity and specificity of the metrics are very different and, in some cases, strongly depends on the acquisition protocol the impact of which is found to be different than assumed in the literature. In any case, NODDI-derived metrics related to the intra-axonal volume fraction (over the whole voxel) - fia - and isotropic volume fraction - fiso - show the best performances in terms of sensitivity with respect to both demyelination and axonal loss, especially when the optimized acquisition protocols are used. Simulation results also confirm the recent experimental observations demonstrating that NODDI-derived fia parameter provides the unique opportunity to directly estimate with a good accuracy, the true intra-axonal volume fraction by using a non-invasive and clinically feasible

DWI technique.
It is indeed possible to extract information on brain microstructure by using a non-invasive imaging method but it is not possible to distinguish between demyelination and early axonal loss with ordinary clinical acquisition protocols. NODDI-derived intra-axonal fractional volume is the best candidate to become a potential biomarker in demyelinating diseases. Special care is recommended in selecting the acquisition protocol: it has a non-negligible impact on the sensitivity and specificity of the metrics.

Disclosures: C. Del Gratta: None. **S. Oliviero:** None.

Poster

092. Connectomics Analytics II

Location: Hall A

Time: Saturday, October 19, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 092.30/BB81

Topic: D.07. Vision

Support: NIH Grant R24MH114799

Title: DotMotif: Subgraph isomorphisms on large brain graphs

Authors: *J. MATELSKY, E. C. JOHNSON, E. P. REILLY, W. R. GRAY RONCAL; Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: Recent advances in neuroscience and bioimaging have enabled scientists to explore brain structure at the level of individual nanoscale synaptic connections. This measure of connectivity, when represented as **a graph with neurons as nodes and synapses as directed edges**, can be large and complex, presenting significant barriers to searching for structure and testing neural-circuit hypotheses. **We leverage graph database and analysis libraries to provide an easy-to-use grammar suitable for rapidly constructing queries and searching for subgraph isomorphisms**, or "motifs." This abstracts many of the computer science and graph theory challenges associated with nanoscale connectomics and allows neuroscientists to quickly achieve reproducible findings.
Our approach, dubbed "*DotMotif*," simplifies identification of basic as well as complex neural connectivity structures relevant to the systems-neuroscience community. We contextualize these results and demonstrate efficiently that the found motifs are unlikely to have occurred by chance; we also demonstrate the versatility of our approach through the exploration of errorful graphs. All of **our tools are released open source** to empower other scientists to use and extend these methods.

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